Fetal death: classification and diagnostic work-up

Fleurisca J. Korteweg

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‘Not everything that is faced can be changed. But nothing can be changed until it is faced’

James Arthur Baldwin

Aan mijn ouders

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General introduction and outline of the thesis

Fetal death

Fetal death or stillbirth is a major obstetrical complication and a devastating experience for parents and caregivers. For bereaved parents it is among the most stressful life events and it is not seldom that they show acute emotional distress and symptoms of depression.[1] Stillbirth is defined as the delivery of a baby showing no signs of life as indicated by the absence of breathing, heartbeats, pulsation of the umbilical cord, or definite movements of voluntary muscles. Fetal death, the largest subgroup of perinatal mortality worldwide consists of intrauterine antepartum fetal deaths (IUFD) and intrapartum fetal deaths.

There is not complete uniformity internationally with regard to birth weight and gestational age criteria for reporting fetal death and perinatal mortality as a whole. In 1992, the WHO introduced the 10th revision of the International Classification of diseases (ICD-10) which defines for perinatal mortality the period commencing at 22 completed weeks of gestation (birth weight is normally about 500 grams) and ending 7 days postnatally.[2] However, the suggested requirement is to report intrauterine fetal deaths at 20 weeks of gestation or greater, or a weight greater than or equal to 350 grams if the gestational age is unknown.[3]

Worldwide an estimate of at least 3.2 million stillbirths occur each year.[4] The majority of these deaths occur in developing countries. In developed countries approximately 1 in 200 pregnancies ends in stillbirth. In the US approximately 25.000 stillbirths are reported annually.[5] For the Netherlands this is 1200-1400 stillborn babies ≥ 22 weeks of gestation.[6] This is five times higher than deaths due to the sudden infant death syndrome and nearly double the number of lethal traffic accidents in the Netherlands. Of these stillborn babies 900-1200 are intrauterine antepartum fetal deaths.[6]

There has been no reduction in the intrauterine fetal death rate over the past 20 years. While neonatal death and intrapartum fetal death rates have continued to steadily decline with improvements in care, antepartum fetal death has emerged as the leading category of perinatal mortality.[7] Many IUFDs occur unexpectedly towards the end of pregnancy.

Peristat is a perinatal monitoring programme initiated by the European Committee to benchmark perinatal mortality between European countries. The Peristat studies showed that perinatal mortality above 22 weeks of gestation, especially fetal mortality, was substantially higher in the Netherlands when compared to other European countries.[8] In Peristat-I the Netherlands had the highest fetal mortality rate (7.4 per 1000 total number of births). In Peristat-II after France, the Netherlands had the second highest fetal mortality rate (7.0 per 1000 total number of births).[9] The Netherlands has a relatively high number of home births but these do not seem to increase the risk of perinatal mortality and severe perinatal morbidity

among low-risk women if the maternity care system facilitates this choice through the availability of well-trained midwives and a good transportation and referral system.[10] The Netherlands has a relatively high number of older mothers and multiple pregnancies, both only partly explain the high Dutch perinatal mortality rate.[9] In addition, Dutch parents make less use of prenatal diagnosis and subsequent termination of pregnancy for congenital anomalies while Dutch neonatologists are more likely to refrain from treating very preterm newborns if their prospects are unfavourable. The Peristat group ended their conclusion with an advice for a more prominent position for perinatal health and the quality of perinatal healthcare in Dutch research programmes.

Fetal death is an under-recognised and under-researched public health problem. Health care providers are responsible for providing support to parents and their families and for investigating the cause of fetal death. Although many risk factors have been identified unfortunately the cause of death remains unexplained in about two-thirds of cases.[11-13] Efforts to address this problem are limited by the lack of information on causes of death. Underpinning this lack of information is that there is internationally no consensus regarding classification of cause and diagnostic investigations into causes of fetal death. In most cases, fetal death certificates are filled out before a full postmortal investigation is performed, and amended death certificates are rarely filled when additional information from the fetal death evaluation emerges.

Classification of cause of perinatal mortality

While there will always be a degree of uncertainty about whether any particular perinatal death was actually caused by a particular condition, there are intensified demands on medical, political and epidemiological grounds for proper determination and classification of cause of perinatal death. This is essential for parents in their process of mourning and to alleviate feelings of guilt, which parents often experience. It can also give parents and caregivers insight into why it happened. Determination of a cause is needed in order to be able to ascertain the recurrence risk and for aiding counselling for future pregnancies, siblings and families. In addition it enables comparison of national and international health care and aids prevention and future research.[8,14-16] Even when a cause of death is not identified exclusion of other causes is also valuable.

Classification of perinatal mortality is complex due to the complicated pathophysiological processes encountered in the mother, fetus and placenta, and as a result of their interaction.[17] Often there is a complex chain of events preceding death. The multiplicity of contributing factors and the different background of the clinicians involved, adds to the confusion.

More than thirty classification systems for perinatal mortality have been introduced since 1954.[18-46] Different classification systems have been designed for diverse reasons with different purposes, approaches, definitions, levels of complexity and availability of guidelines. These systems have differing categories for classifying causes and varying definitions for relevant conditions. Clear uniform definitions and classification guidelines make a model easy to use and uni-interpretable.[32,44] However, definitions of cause of death categories and guidelines are incomplete or not described in more than half of the systems.[18-21,27,28,31,33,34,36-38,41-43,45,46] As a result, no single classification system is universally accepted and each has strengths and weaknesses.

Guideline for investigation of fetal death

While the approach to classification is partly responsible for inadequately investigated fetal deaths, the level of investigation also plays an important role. The value of any classification system is primarily dependent on identifying and collecting all important information for each mortality case. This is best achieved through a systematic approach to diagnostic investigation or work-up and review of findings in the context of the clinical setting in which the death occurred. Such protocols increase and enhance the diagnostic accuracy and consistency of the investigation process. The purpose of a formal stillbirth investigation guideline is to guide health professionals in the stillbirth investigation process and to provide information concerning the cause of death. The proportion of unexplained stillbirths is lower in centres that conduct a systematic and well defined evaluation for causes of stillbirth.[47] However, in many studies of investigation protocols, still a large proportion of stillbirths remain unexplained ranging from 36% to 60%.[48-50] Due to limitations in current research and the complexity of the issue, the optimal workup after fetal death is unknown. Both internationally and in the Netherlands there is no uniform evidence-based workup guideline after fetal death. Local protocols have often been designed on expert opinion; they differ and are extensive. This brings along high costs and a strain for parents. The value of many commonly used diagnostic tests for determination of cause of fetal death is unclear. Consequently, there is discussion about which tests and examinations should be included in a routine investigative workup to ensure an acceptable chance of determination of the cause of fetal death. A comparison of the components of currently used protocols identified wide variation. The authors of a recent review concluded that autopsy and placental pathology were valuable. Due to a lack of high quality data on the value of other investigations, no formal scientific judgement could be made on which is the most appropriate guideline for stillbirth investigations, or which components should be considered for the most relevant and efficient investigative

protocol.[51] The aim of investigation is to optimise diagnostic accuracy while limiting the burden of testing for women so soon after the tragedy of stillbirth.
In current practice, the majority of stillbirths are inadequately investigated and therefore important information may often be missed. We demonstrated earlier that up to 50% of diagnostic test results after fetal death are incomplete or missing, resulting in disappointment, frustration and emotional burden for parents and caregivers.[52]

ZOBAS study

In 2002 we initiated the ZOBAS (Zinnig Onderzoek Bij Antepartum Sterfte) study. This is a prospective cohort study investigating the value of diagnostic tests after intrauterine antepartum fetal death for determination of cause of death. This study was performed in 50 secondary and tertiary referral hospitals in the Netherlands (Appendix 1: ZOBAS participating hospitals), serving rural as well as urban populations from 2002 to 2008. Inclusion criteria were singleton intrauterine fetal deaths diagnosed antepartum (heartbeat ceased before labour) after 20 weeks of gestation. Pregnancy terminations and intrapartum deaths were excluded. A total of 1025 intrauterine antepartum fetal deaths were included. The study was approved by the review boards of all hospitals and written informed consent was obtained from all participants. Each couple whose fetus died, was managed in the same way. Data were collected for each intrauterine antepartum fetal death, including medical and obstetric history, maternal and fetal characteristics, and pregnancy and birth details (Appendix 2: case record form). Our diagnostic work-up protocol was based on currently used local protocols and diagnostics were included if most Dutch hospitals performed these tests after fetal death (Appendix 3: diagnostic flowchart). The protocol included: maternal blood tests including full blood count, chemistry and viral serology; coagulation tests for couples performed centrally in the laboratory in Groningen; fetal blood tests including viral serology; microbiological cultures from the mother, fetus and placenta; autopsy; placental examination (Appendix 4: pathology protocol); and cytogenetic analysis. Multidisciplinary panel classification sessions were set up for determination of cause of all fetal deaths and the value of diagnostics in this determination.

Outline of this thesis

The studies in this thesis discuss the dilemmas around classification of the cause of perinatal mortality and diagnostic work-up after intrauterine antepartum fetal death to determine the cause of death. *Part I -Classification of perinatal mortality* focuses on diverse aspects of different classification systems for perinatal mortality. *Part II - Value of diagnostic tests after intrauterine antepartum fetal death* focuses on different causes of fetal death, their clinical manifestations and the value of different diagnostic tests in allocating an underlying cause of death. *Part III - Fetal death workup guideline* describes a proposal for fetal death diagnostic work-up after evaluation of the ZOBAS cohort.

Part I

Classification of perinatal mortality

Classification of perinatal mortality has been a topic of interest for several decades. No national or international consensus has been achieved on which system to use. In Chapter 1 a newly developed classification system, the Tulip classification is discussed which separates cause, mechanism, origin of mechanism and contributing factors of perinatal mortality for the purpose of counselling and prevention. The goal was to propose a well defined, unambiguous, single cause system aiming to identify the initial demonstrable pathophysiological entity initiating the chain of events that has irreversibly led to death, based on the combination of clinical findings and diagnostic test results including pathological findings. In Chapter 2, use of the Tulip classification for allocation of cause of fetal death is compared to other currently used international classification systems. The focus is on placental causes of death and whether information was gained or lost by classification in the different systems because this could have consequences for counselling parents, targeting research and preventive strategies, and for the validity of statistics. In Chapter 3 existing classification systems are compared regarding their definition of the perinatal period, level of complexity, inclusion of maternal, fetal and/or placental factors and whether they focus on a clinical or pathological viewpoint. This led to proposal of a systematic multilayered approach for the analysis of perinatal mortality using one or more of the previously published classification systems.

Part II

Value of diagnostic tests after intrauterine antepartum fetal death

A second topic that will be discussed in this thesis is the value of diagnostic tests after intrauterine antepartum fetal death for allocation of different causes of death. A large cause of death category is placental pathology. The value of placental examination and the occurrence of different placental causes related to different gestational age periods, and their clinical manifestations during pregnancy in the ZOBAS cohort are described in Chapter 4. One of these placental causes is the relatively unknown villus immaturity causing unexpected fetal death after 36 weeks of gestation. The prevalence and clinico-pathological associations of this entity are described in Chapter 5. A substantial proportion of intrauterine antepartum fetal deaths are caused by genetic abnormalities. Criteria for investigation into chromosomal abnormalities after fetal death differ internationally with recommendation of different techniques and different groups to be tested ranging from testing all fetal deaths to a select group. In Chapter 6, success rates are estimated for cytogenetic analysis in different types of tissue after fetal death in the ZOBAS cohort. In addition, selection criteria for cytogenetic analysis are studied and the value of this test for determination of the cause of fetal death. This led to recommendations for a fetal death cytogenetic flowchart. Maternal inherited thrombophilic defects are recognized as risk factors for pregnancy complications such as severe pre-eclampsia, placental abruption, intrauterine growth restriction and fetal death. However, this has not been demonstrated consistently. In a retrospective family cohort study (Descartes study) of women with hereditary deficiencies of either antithrombin, protein C and S, the absolute risk of fetal death comparing deficient women to non-deficient female relatives was calculated and the contribution of additional thrombophilic defects to this risk (Chapter 7). The pathophysiology of fetal death associated with thrombophilia is presumed to be thrombosis in the uteroplacental circulation. Although this association remains uncertain this has resulted in routine thrombophilia work-up after fetal death in many hospitals. In Chapter 8 prevalence of maternal thrombophilic defects, either acquired or inherited, and paternal thrombophilic defects in the ZOBAS cohort were compared to prevalence in the normal population. Furthermore, the association between these thrombophilic defects and the various causes of fetal death within this cohort was assessed.

Part III

Fetal death workup guideline

There is no international golden standard fetal death work-up guideline. This limits investigation into causes. Chapter 9 describes identification of valuable tests for determining the cause of intrauterine antepartum fetal death by a multidisciplinary evaluation of diagnostic procedures performed prospectively in the ZOBAS cohort. This led to recommendations for a basic and selective workup guideline for fetal death.

Finally, the results of the studies are summarized in English and Dutch and in the 'general discussion and future perspectives' section currently ongoing and new research developments in the field of fetal death classification, investigation and prevention are discussed.

References

1. Murray J, Callan VJ. Predicting adjustment to perinatal death. Br J Med Psychol. 1988;61:237-244.
2. World Health Organisation (WHO). International Classification of Diseases, 10th ed. 1992. Geneva.
3. National Center for Health Statistics. Model state vital statistics act and regulations. 1994. Hyattsville, USA.
4. Stanton C, Lawn JE, Rahman H, Wilczynska-Ketende K, Hill K. Stillbirth rates: delivering estimates in 190 countries. Lancet. 2006;367:1487-1494.
5. Macdorman MF, Munson ML, Kirmeyer S. Fetal and perinatal mortality, United States, 2004. Natl Vital Stat Rep. 2007;56:1-19.
6. the Netherlands Perinatal Registry. Doodgeborenen (intra uteriene vruchtdood en intra partum sterfte) per 1000 geborenen na een zwangerschapsduur van 22 weken van 2002 tot 2007. 2009. Amsterdam
7. Fretts RC, Boyd ME, Usher RH, Usher HA. The changing pattern of fetal death, 1961-1988. Obstet Gynecol. 1992;79:35-39.
8. Buitendijk S, Zeitlin J, Cuttini M, Langhoff-Roos J, Bottu J. Indicators of fetal and infant health outcomes. Eur J Obstet Gynecol Reprod Biol. 2003;111 Suppl 1:S66-S77.
9. Mohangoo AD, Buitendijk SE, Hukkelhoven CW et al. Higher perinatal mortality in The Netherlands than in other European countries: the Peristat-II study. Ned Tijdschr Geneeskd. 2008;152:2718-2727.
10. de Jonge A, van der Goes BY, Ravelli AC et al. Perinatal mortality and morbidity in a nation-wide cohort of 529,688 low-risk planned home and hospital births. BJOG. 2009;116:1177-1184.
11. Fretts RC. Etiology and prevention of stillbirth. Am J Obstet Gynecol. 2005;193:1923-1935.
12. Goldenberg RL, Kirby R, Culhane JF. Stillbirth: a review. J Matern Fetal Neonatal Med. 2004;16:79-94.
13. Silver RM. Fetal death. Obstet Gynecol. 2007;109:153-167.
14. Galan-Roosen AE, Kuijpers JC, van der Straaten PJ, Merkus JM. Evaluation of 239 cases of perinatal death using a fundamental classification system. Eur J Obstet Gynecol Reprod Biol. 2002;103:37-42.
15. Gardosi J, Kady SM, McGeown P, Francis A, Tonks A. Classification of stillbirth by relevant condition at death (ReCoDe): population based cohort study. BMJ. 2005;331:1113-1117.
16. Kramer MS, Liu S, Luo Z, Yuan H, Platt RW, Joseph KS. Analysis of perinatal mortality and its components: time for a change? Am J Epidemiol. 2002;156:493-497.
17. Wigglesworth JS. Causes and classification of fetal and perinatal death. Fetal and perinatal pathology. London: Blackwell science; 1998:75-86.
18. Alberman E, Botting B, Blatchley N, Twidell A. A new hierarchical classification of causes of infant deaths in England and Wales. Arch Dis Child. 1994;70:403-409.
19. Alberman E, Blatchley N, Botting B, Schuman J, Dunn A. Medical causes on stillbirth certificates in England and Wales: distribution and results of hierarchical classifications tested by the Office for National Statistics. Br J Obstet Gynaecol. 1997;104:1043-1049.

20. Alessandri LM, Chambers HM, Blair EM, Read AW. Perinatal and postneonatal mortality among Indigenous and non-Indigenous infants born in Western Australia, 1980-1998. Med J Aust. 2001;175:185-189.

21. Autio-Harmainen H, Rapola J, Hoppu K, Osterlund K. Causes of neonatal deaths in a pediatric hospital neonatal unit. An autopsy study of a ten-year period. Acta Paediatr Scand. 1983;72:333-337.

22. Baird D, Walker J, Thomson AM. The causes and prevention of stillbirths and first week deaths. III. A classification of deaths by clinical cause; the effect of age, parity and length of gestation on death rates by cause. J Obstet Gynaecol Br Emp. 1954;61:433-448.

23. Bound JP, Butler NR, Spector WG. Classification and causes of perinatal mortality. Br Med J. 1956;12:1191-1196.

24. Bound JP, Butler NR, Spector WG. Classification and causes of perinatal mortality. II. Br Med J. 1956;44:1260-1265.

25. Butler NR, Alberman ED. Perinatal problems: the second report of the 1958 British Perinatal Mortality Survey. 1969. Edinburgh, E & S Livingstone LtD.

26. Butler NR, Bonham DG. Perinatal mortality: the first report of the 1958 British Perinatal Mortality Survey. 1963. Edinburgh, E & S Livingstone Ltd.

27. Chan A, King JF, Flenady V, Haslam RH, Tudehope DI. Classification of perinatal deaths: development of the Australian and New Zealand classifications. J Paediatr Child Health. 2004;40:340-347.

28. Chang A, Keeping JD, Morrison J, Esler EJ. Perinatal death: audit and classification. Aust N Z J Obstet Gynaecol. 1979;19:207-211.

29. Cole S, Hartford RB, Bergsjo P, McCarthy B. International collaborative effort (ICE) on birth weight, plurality, perinatal, and infant mortality. III: A method of grouping underlying causes of infant death to aid international comparisons. Acta Obstet Gynecol Scand. 1989;68:113-117.

30. Cole SK, Hey EN, Tomson AM. Classifying perinatal death: an obstetric approach. Br J Obstet Gynaecol. 1986;93:1204-1212.

31. Fairweather DV, Russell JK, Anderson GS, Bird T, Millar DG, Pearcy PA. Perinatal mortality in Newcastle upon Tyne 1960-62. Lancet. 1966;1:140-142.

32. Galan-Roosen AE, Kuijpers JC, van der Straaten PJ, Merkus JM. Fundamental classification of perinatal death. Validation of a new classification system of perinatal death. Eur J Obstet Gynecol Reprod Biol. 2002;103:30-36.

33. Hey EN, Lloyd DJ, Wigglesworth JS. Classifying perinatal death: fetal and neonatal factors. Br J Obstet Gynaecol. 1986;93:1213-1223.

34. Hovatta O, Lipasti A, Rapola J, Karjalainen O. Causes of stillbirth: a clinicopathological study of 243 patients. Br J Obstet Gynaecol. 1983;90:691-696.

35. Keeling JW, MacGillivray I, Golding J, Wigglesworth J, Berry J, Dunn PM. Classification of perinatal death. Arch Dis Child. 1989;64:1345-1351.

36. Knutzen VK, Baillie P, Malan AF. Clinical classification of perinatal deaths. S Afr Med J. 1975;49:1434-1436.

37. Lammer EJ, Brown LE, Anderka MT, Guyer B. Classification and analysis of fetal deaths in Massachusetts. JAMA. 1989;261:1757-1762.

38. Langhoff-Roos J, Borch-Christensen H, Larsen S, Lindberg B, Wennergren M. Potentially avoidable perinatal deaths in Denmark and Sweden 1991. Acta Obstet Gynecol Scand. 1996;75:820-825.

39. Low JA, Boston RW, Cervenko FW. A clinical classification of the mechanisms of perinatal wastage. Can Med Assoc J. 1970;102:365-368.

40. Low JA, Boston RW, Crussi FG. Classification of perinatal mortality. Can Med Assoc J. 1971;105:1044-1046.

41. McIlwaine GM, Howat RC, Dunn F, Macnaughton MC. The Scottish perinatal mortality survey. Br Med J. 1979;2:1103-1106.

42. Morrison I, Olsen J. Weight-specific stillbirths and associated causes of death: an analysis of 765 stillbirths. Am J Obstet Gynecol. 1985;152:975-980.

43. Naeye RL. Causes of perinatal mortality in the U.S. Collaborative Perinatal Project. J Am Med Assoc. 1977;238:228-229.

44. Whitfield CR, Smith NC, Cockburn F, Gibson AA. Perinatally related wastage--a proposed classification of primary obstetric factors. Br J Obstet Gynaecol. 1986;93:694-703.

45. Wigglesworth JS. Monitoring perinatal mortality. A pathophysiological approach. Lancet. 1980;2:684-686.

46. Winbo IG, Serenius FH, Dahlquist GG, Kallen BA. NICE, a new cause of death classification for stillbirths and neonatal deaths. Neonatal and Intrauterine Death Classification according to Etiology. Int J Epidemiol. 1998;27:499-504.

47. Petersson K, Bremme K, Bottinga R et al. Diagnostic evaluation of intrauterine fetal deaths in Stockholm 1998-99. Acta Obstet Gynecol Scand. 2002;81:284-292.

48. Incerpi MH, Miller DA, Samadi R, Settlage RH, Goodwin TM. Stillbirth evaluation: what tests are needed? Am J Obstet Gynecol. 1998;178:1121-1125.

49. Lim TL, Tan KH, Tee CS, Yeo GS. Investigating stillbirths using a simplified obstetric events-based protocol. Singapore Med J. 2005;46:63-68.

50. Pauli RM, Reiser CA. Wisconsin Stillbirth Service Program: II. Analysis of diagnoses and diagnostic categories in the first 1,000 referrals. Am J Med Genet. 1994;50:135-153.

51. Corabian P, Scott NA, Lane C, Guyon G. Guidelines for investigating stillbirths: an update of a systematic review. J Obstet Gynaecol Can. 2007;29:560-567.

52. Holm JP, Duyndam DAC, Erwich JJHM. Intra-uterine vruchtdood zonder duidelijke oorzaak: zinnig onderzoek, onderzoek zinnig? 1996. NTOG;109:373-377.

Part I

Classification of perinatal mortality

Chapter 1

The Tulip classification of perinatal mortality: introduction and multidisciplinary inter-rater agreement

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ABSTRACT

Objective

To introduce the pathophysiological Tulip classification system for underlying cause and mechanism of perinatal mortality based on clinical and pathological findings for the purpose of counselling and prevention.

Design

Descriptive.

Setting

Tertiary referral teaching hospital.

Population

Perinatally related deaths.

Methods

A classification consisting of groups of cause and mechanism of death was drawn up by a panel through the causal analysis of the events related to death. Individual classification of cause and mechanism was performed by assessors. Panel discussions were held for cases without consensus.

Main outcome measures

Inter-rater agreement for cause and mechanism of death.

Results

The classification consists of six main causes with subclassifications: 1. congenital anomaly (chromosomal, syndrome, single or multiple organ system), 2. placenta (placental bed, placental pathology, umbilical cord complication, not otherwise specified [NOS]), 3. prematurity (preterm prelabour rupture of membranes, preterm labour, cervical dysfunction, iatrogenous and NOS), 4. infection (transplacental, ascending, neonatal and NOS), 5. other (fetal hydrops of unknown origin, maternal disease, trauma and out of the ordinary) and 6. unknown. Overall kappa coefficient for agreement for cause was 0.81 (95% C.I. 0.80-0.83). Six mechanisms were drawn up: cardio/circulatory insufficiency, multi-organ failure, respiratory insufficiency, cerebral insufficiency, placental insufficiency and unknown. Overall kappa for mechanism was 0.72 (95% C.I. 0.70-0.74).

Conclusions

Classifying perinatal mortality to compare performance over time and between centres is useful and necessary. Interpretation of classifications demands consistency. The Tulip classification allows unambiguous classification of underlying cause and mechanism of perinatal mortality, gives a good inter-rater agreement, with a low percentage of unknown causes, and is easily applicable in a team of clinicians when guidelines are followed.

INTRODUCTION

There are intensified demands on medical, political and epidemiological grounds for proper determination and classification of cause of perinatal mortality.[1-4] Such classification is complex due to the complicated pathophysiological processes encountered in the mother, fetus and placenta, and as a result of their interaction.[5] The multiplicity of contributing factors and the different background of the clinicians involved add to the confusion.

Thirty classification systems for perinatal mortality have been introduced since 1954.[6-34] Systems have been designed for different reasons with different approaches, definitions and levels of complexity. Twenty systems focus on either pathological information or on clinical details,[6,7,9-12,14-16,18,21,23,24,29-34] whereas in our opinion both should be considered for classification. Half the systems aim at classifying the underlying cause of death.[6-8,10,13,15,18,20,29-32,34] Systems should not confuse this underlying cause of death with mechanism of death and risk factors.[3] Some systems are brief and easy to use, others are more detailed. Preferably, classification systems should contain a structure that allows unambiguous allocation to representative cause-of-death groups to ensure a high percentage of cases classified with a known cause of death.[20] It should be possible to amend a system to allow for future scientific developments without disturbing the system.[4] Clear uniform definitions and classification guidelines make a model easy to use and uni-interpretable.[20,32] However, definitions of cause-of-death categories and guidelines are incomplete or not described in more than half of the articl es.[6-9,15,16,19,21,22,24-26,29-31,33,34] Definitions of the perinatal period change over time and are not always unanimous between centres.[21,35-37] There is need for a system that permits classification of cases occurring during the complete perinatal period independent of the used definitions.

Classification of cause of death must be independent of the speciality of the clinician.[23] It is important that there be a good inter-rater agreement and that classifications used are reproducible.[18,21,23,38] Only some systems test their level of agreement. This inter-rater agreement varies from 0.50-0.59 measured by independent raters[38] to 0.85-0.90 determined by the original assessors themselves.[15] The mother, the fetus and the placenta are all involved in the complex process of perinatal mortality; they should be addressed together. Only two systems consider these three factors together.[20,22] However, de Galan-Roosen et al.[20] have minimal subclassification of the placenta group, and the classification of Hovatta et al[22] is designed for the stillbirth group only. Our view was that existing classification systems for perinatal mortality did not fulfil our needs.

Our objective was to develop a new classification system that separates cause and mechanism of perinatal mortality for the purpose of counselling and prevention.

Our goal was to propose a well-defined, unambiguous, single-cause system aiming to identify the initial demonstrable pathophysiological entity initiating the chain of events that has irreversibly led to death, based on the combination of clinical findings and diagnostic test results, including pathological findings. We describe here and assess the inter-rater agreement of the pathophysiological Tulip classification for cause and mechanism of perinatal mortality in a multidisciplinary setting.

METHODS

To design a pathophysiological classification system for perinatal mortality, a panel of three obstetricians, a pathologist, a neonatologist, a clinical geneticist and two obstetrical residents organised panel meetings. The system was named Tulip as this is a well-known Dutch association. First, cause of death was defined as the initial, demonstrable pathophysiological entity initiating the chain of events that has irreversibly led to death. The mechanism of death was defined as the organ failure that is not compatible with life, initiated by the cause of death that has directly led to death. Origin of mechanism was defined as the explanation of the mechanism of death. This third step of the classification was proposed to make the pathway of death more clear and to prevent confusion with cause of death. The system was designed to include late fetal losses, stillbirths, early neonatal deaths, late neonatal deaths and perinatally related infant deaths during hospital admission from birth onwards.

Then we decided whether a strict hierarchy would be preferable for the system as hierarchy makes use easier. During multidisciplinary panel sessions, we proposed the concept that the cognitive process involved in making explicit the complex process of integrating all possible information to allocate the underlying cause and mechanism of death is comparable with diagnostic reasoning in clinical medicine, which has been described by other disciplines.[39] Since diagnostic reasoning is differential diagnosis and pattern recognition driven rather than hierarchical, we concluded that our classification system for underlying cause of death could not be strictly hierarchical.

The six main groups of causes of death with subclassifications, and the mechanisms of death were developed by the panel according to the causal analysis of 109 perinatally related deaths during a one-year period. Case notes and results of complete diagnostic work-up (as current at that time in our institution) were available. Discussions between panel members on the basis of information from existing classifications and current obstetrical, pathologic, neonatologic and genetic literature on causes of perinatal mortality led us to the Tulip system. As

congenital anomalies and placental pathology represent major causes of perinatal mortality, we decided to design detailed subclassifications for these groups.
Table 1 shows the categories for cause of death, and Table 2 shows the categories for mechanism of death. Definitions for the terms used and allocation to a certain category, as well as examples of clinical pathological entities, were drawn up in a guideline.

Tulip guideline

(1) ***Congenital anomaly***: the cause of death is explained by a genetic or structural defect incompatible with life or potentially treatable but causing death. Assignment to this group is justified if the congenital anomaly is the actual cause of death and no other major categories of causes of death has initiated the causal pathway leading to death. Termination of pregnancy because of a congenital anomaly is also classified in the group; subclassification is dependent on the defect. These include *chromosomal defects* (1.1) with subclassification by type, *syndromal* (1.2) with subclassification by whether monogenic or not and organ specific abnormalities such as *central nervous system* (1.3) or *heart and circulatory system* (1.4). Examples are shown in Table 1.
(2) ***Placenta:*** the cause of death is explained by a placental pathological abnormality supported by the clinical findings. (2.1) *Placental bed pathology:* inadequate spiral artery remodelling and/or spiral artery pathology leading to uteroplacental vascular insufficiency such as placental infarction. (2.2) *Placental pathology:* pathology originated during development of the placenta itself, abnormalities in the parenchyma or localisation of the placenta. (2.2.1) *Development:* morphologic abnormalities that arise because of abnormal developmental processes such as placenta circumvallata, villus immaturity and placenta hypoplasia. (2.2.2) *Parenchyma:* acquired placenta parenchyma disorders of the villi or intervillous space. Examples are villitis of unknown origin, massive perivillous fibrin deposition and fetomaternal haemorrhage, without obvious cause. (2.2.3) *Abnormal localisation:* example is placenta praevia. (2.3) *Umbilical cord complication:* acquired umbilical cord complications supported by clinical findings. Example is umbilical cord prolapse, with occlusion of the vessels. (2.4*) Not otherwise specified:* the cause of death falls into the group placenta, but because of the existence of different placenta subclassifications, a choice cannot be made as to what was first in the chain of events leading to death.
(3) ***Prematurity/immaturity:*** the cause of death is explained by the initiation of preterm delivery only and in the case of neonatal death also, with the associated problems of prematurity/ immaturity. (3.1) *Preterm prelabor rupture of membranes (PPROM)* initiates preterm delivery. (3.2) *Preterm labor* where uterus contractions initiate preterm delivery. (3.3) *Cervical dysfunction* initiates preterm delivery.

(3.4) *Iatrogenic* procedures initiates preterm delivery on maternal non-obstetrical indication only, for example caesarean section on maternal indication for carcinoma. (3.5) *NOS* where prematurity/ immaturity is the cause of death but it is not clear how preterm delivery was initiated.
(4) ***Infection:*** the cause of death is explained by an infection resulting in sepsis and stillbirth or neonatal death. There is clear microbiological evidence of infection with matching clinical and pathological findings. (4.1) *Transplacental* where there is a haematogenous infection through the spiral arteries, the placenta and the umbilical cord to the fetus such as Parvovirus infection. (4.2) *Ascending* where there is an ascending infection from colonisation of the birth canal such as Streptococci group B infection. (4.3) *Neonatal* where there is infection acquired after birth such as Escherichia coli sepsis-meningitis. (4.4) *NOS* where there is infection, but it cannot be discerned whether the infection was transplacental, ascending or acquired after birth.
(5) ***Other:*** the cause of death is explained by another specific cause not mentioned in the previous groups of cause of death. (5.1) *Fetal hydrops of unknown origin.* (5.2) *Maternal disease* is severe enough to jeopardise the fetus or the neonate, initiating death. Examples might be severe maternal sepsis or alloimmunisation. For most maternal medical conditions this classification (5.2) will only apply when the disease leads directly to perinatal death, as in diabetic ketoacidosis. Otherwise, the condition is a risk factor. (5.3) *Trauma.* (5.3.1) *Maternal* such as severe road traffic accidents. (5.3.2) *Fetal* such as birth trauma. (5.4) *Out of the ordinary:* a specific event or condition initiating the causal pathway to fetal or neonatal death such as rupture of the uterus.
(6) ***Unknown.*** (6.1) *Despite thorough investigation.* (6.2) *Important information missing.*
To register more information about each case of perinatally related mortality, it is also possible to describe *contributing factors,* defined as other known factors on the causal pathway to death e.g. risk factors such as obesity and smoking, and *comorbidity*, defined as an event or condition relevant for the clinical situation or the care given but not part of the causal pathway to death. Case examples illustrating use of the Tulip classification are shown in the Appendix.

Agreements on cause, mechanism of death and origin of mechanism

Because certain case situations led to discussions, an additional list of agreements for cause, mechanism of death and origin of mechanism for use in our centre were prepared beforehand.
(1) If a pregnancy was terminated with prostaglandins for a congenital anomaly, the congenital anomaly was considered the cause of death, placental insufficiency

the mechanism of death and induction was the origin of mechanism. If a fetus was born alive after this procedure and died within hours, respiratory insufficiency was considered as the mechanism of death and induction the origin of mechanism.
(2) In the case of a sequence of recurrent vaginal blood loss, PPROM and a placenta circumvallata, we considered developmental placental pathology (2.2.1) as the cause of death.
(3) If cause of intrauterine death was developmental placental pathology (2.2.1) due to a twin-to-twin-transfusion syndrome, cardiocirculatory insufficiency was considered as the mechanism of death for both the donor and the recipient fetus.
(4) If a fetus died due to umbilical cord prolapse, the mechanism of death was cardiocirculatory insufficiency.
(5) If treatment was not initiated after birth for a nonviable, very early preterm neonate, respiratory insufficiency was considered as the mechanism of death and prematurity as origin of mechanism.
(6) If intrauterine fetal death was attributable to infection, multi-organ failure was considered the mechanism of death and intrauterine infection the origin of mechanism. In the case of neonatal death due to infection, multi-organ failure was considered the mechanism of death and sepsis the origin of mechanism.
(7) If intrauterine fetal death was due to fetal hydrops of any cause, cardiocirculatory insufficiency could only be considered as mechanism of death if a hyperdynamic circulation existed.
(8) Important information missing was defined as two out of three diagnostic investigations missing regarding pathological examination: autopsy and placental examination, chromosomal or microbiological investigation.

Origin of mechanism

Cessation of treatment for origin of mechanism is eligible when there is a medical prognosis of either early death (for example, Potters syndrome) or severe impairment associated with a very poor quality of life (for example, neurological damage due to severe asphyxia, congenital anomalies).[40] Cessation of treatment is not the origin of mechanism if the death was imminent. In the case of cessation of treatment of the neonate by reason of very poor prognosis, mechanism of death allocated was respiratory insufficiency.

Inter-rater agreement

After design of the Tulip classification system a panel consisting of the original assessors who developed the system assessed the inter-rater agreement of the system for cases of perinatal mortality occurring during the four year period of 1999-2002. During this period, there were 7389 total births (stillborn and liveborn

> 16 weeks of gestation) at our institution. A retrospective analysis was performed on all perinatally related deaths occurring during this period. These deaths comprised late fetal losses (spontaneous fetal loss and termination of pregnancy from 16 completed weeks of gestation until 22 weeks of gestation). Perinatally related deaths beyond 22 weeks of gestation were defined as stillbirths, early neonatal deaths (death up to 7 completed days after birth), late neonatal deaths (death from 8 up to 28 completed days after birth) and perinatally related infant deaths (death from 29 days up to 6 completed months after birth during hospital admission from birth onwards).

Two independent researchers compiled narratives for each mortality case, describing chronologically the most important events. Narratives were based upon medical and obstetric history, information about the pregnancy, diagnostic test results including pathological findings concerning autopsy and placental investigation and obstetric and neonatology discharge letters. No other information sources were consulted.

The panel consisted of two obstetricians, an obstetrical resident, a neonatologist and a pathologist, each of whom individually classified cause and mechanism of death for all cases. Procedures were agreed upon in advance. Only one underlying cause and one mechanism of death could be allocated. Assessors were unaware of the results of classification from other panel members. Second, panel discussions were held for cases without initial consensus on cause or mechanism of death, and after debate, a panel consensus was agreed upon. A panel judgement for origin of mechanism was also allocated. Cases, in which panel members failed to comply with the definitions for allocation to a certain category, stated in the guidelines, were registered as misinterpretation.

Statistical methods

Classification of cause and mechanism of death was performed individually by different assessors. Inter-rater agreement beyond chance between the assessors was calculated using Cohen's kappa. Our qualitative interpretation of the kappa statistic for inter-rater agreement corresponding with others was: < 0.4, poor; 0.40 to <0.55, fair; 0.55 to <0.70, good; 0.70 to <0.85, very good and ≥ 0.85, excellent.[41] Kappa values and 95% confidence intervals (C.I.) were calculated for five assessors.

RESULTS

During the four year period of 1999-2002, there were 411 perinatally related losses, comprising 104 late fetal losses, 153 stillbirths, 108 early neonatal deaths, 25 late

Table 1. Tulip classification of perinatal mortality: causes

Cause of death	n (% of total)	Subclassification		n
1 Congenital anomaly	142	1 Chromosomal defect	1 Numerical	42
	(35)		2 Structural	8
			3 Microdeletion/ uniparental disomy	-
		2 Syndrome	1 Monogenic	15
			2 Other	2
		3 Central nervous system		22
		4 Heart and circulatory system		9
		5 Respiratory system		1
		6 Digestive system		2
		7 Urogenital system		13
		8 Musculoskeletal system		-
		9 Endocrine/metabolic system		-
		10 Neoplasm		2
		11 Other	1 Single organ	-
			2 Multiple organ	26
2 Placenta	111	1 Placental bed pathology		72
	(27)	2 Placental pathology	1 Development	28
			2 Parenchyma	6
			3 Localisation	2
		3 Umbilical cord complication		1
		4 Not otherwise specified		2
3 Prematurity/ Immaturity	95	1 PPROM		52
	(23)	2 Preterm labour		30
		3 Cervical incompetence		12
		4 Iatrogenous		-
		5 Not otherwise specified		1
4 Infection	6	1 Transplacental		2
	(1)	2 Ascending		4
		3 Neonatal		-
		4 Not otherwise specified		-
5 Other	13	1 Fetal hydrops of unknown origin		4
	(3)	2 Maternal disease		5
		3 Trauma	1 Maternal	-
			2 Fetal	-
		4 Out of the ordinary		4
6 Unknown	44	1 Despite thorough investigation		16
	(11)	2 Important information missing		28
Total				411

neonatal deaths and 21 perinatally related infant deaths. The perinatal mortality rate (stillborn and live born > 500 grams, death up to 7 completed days after birth) was 30.7/1000. Clinical records were available for all deaths. An autopsy was performed in 199 (48%) cases and placental examination in 379 (92%). The mean time to individually classify one perinatal death was 15 minutes (range 10-25). Mean time for panel discussions for cases for which there was no consensus was 10 minutes (range 5-20 minutes). Due to experience, discussion time was shortened during the study.

Table 1 shows the distribution of classification of cause of death in the six primary groups of our classification, with further subclassification for the 411 perinatally related deaths. The largest cause-of-death group was *congenital anomalies* and contained 142 cases (35%). A total of 42 (30%) pregnancies were terminated for fetal congenital abnormalities. All terminations were performed before 24 weeks of gestation. Four deaths were classified in the group *other; out of the ordinary*. The first death consisted of a termination of pregnancy at 17 weeks of gestation for an increased risk of congenital anomalies detected with serum screening. The second death was of a neonate who died three days after birth. The child was situated intra-abdominal after a uterus rupture, originating during induction of labour at 42 weeks of gestation. The third case was a neonatal death occurring a few hours after immature labour at 24 weeks of gestation, after recurrent vaginal blood loss due to a cervical polyp. The fourth death was a case of recurrent blood loss after a transcervical chorionic villus biopsy performed at 10 weeks of gestation. The membranes ruptured at 19 weeks of gestation, where after the umbilical cord prolapsed and the fetus died in utero. In 44 cases (11%) the cause of death remained unknown. In 28 (64%) of these deaths, important information was missing.

The perinatally related deaths were distributed among the six different groups of mechanisms (Table 2). Examples of origin of mechanism are presented in Table 3, together with the number of deaths for which we allocated this origin. This table is in contrast to table 1 and 2, not exhaustive and can be modified depending on the pathology involved in the cases being classified.

Table 2. Tulip classification of perinatal mortality: mechanisms

Mechanisms	n	%
1. Cardiocirculatory insufficiency	44	11
2. Multi-organ failure	30	7
3. Respiratory insufficiency	130	32
4. Cerebral insufficiency	7	2
5. Placental insufficiency	123	30
6. Unknown	77	19
Total	411	100

Inter-rater agreement

All 411 deaths were included to calculate the inter-rater agreement for the Tulip classification. In 47% of cases, consensus was achieved for cause of death after individual classification and in 69% of cases after excluding guideline misinterpretations. For mechanism of death, this was in 58% of cases and after

Table 3. Tulip classification of perinatal mortality: examples of origin of mechanism

Origin of Mechanism	n
Cardio-circulatory	
Congenital heart malformation	2
Fetal hydrops	1
Myocardial ischaemia	2
Pneumopericard	1
Supraventriculary tachycardia	1
Twin to twin transfusion	5
Umbilical cord occlusion	14
Pulmonary	
Airway obstruction	2
Bronchopneumonia	1
Chronic Lungdisease/Broncho Pulmonary Dysplasia	9
IRDS/Hyaline membrane disease	11
Lunghypoplasia	25
Placental	
Placental abruption	16
Infarction	24
Villus immaturity/Terminal villus deficiency	4
Hypoplasia	12
Partial mola	2
Fetal thrombotic vasculopathy	3
Massive perivillous fibrin deposition	3
Ectopic placentation	1
Other	
Sepsis	14
Infection intrauterine	12
Prematurity/Immaturity	40
Excessive bleeding	6
Complication after medical procedure	11
Ceasure of treatment	31
Induction	63
Selective feticide	2
None of the above	12
Unknown	81
Total	411

Table 4. Inter-rater agreement over six causes and mechanisms of death by five assessors

Causes	Kappa	95% C.I.
1. Congenital anomaly	0.92	0.89-0.95
2. Placenta	0.83	0.80-0.86
3. Prematurity/Immaturity	0.83	0.80-0.86
4. Infection	0.47	0.44-0.50
5. Other	0.46	0.43-0.49
6. Unknown	0.70	0.67-0.73
Mechanisms		
1. Cardiocirculatory insufficiency	0.58	0.55-0.61
2. Multi-organ failure	0.61	0.58-0.65
3. Respiratory insufficiency	0.83	0.80-0.86
4. Cerebral insufficiency	0.40	0.37-0.43
5. Placental insufficiency	0.78	0.75-0.81
6. Unknown	0.66	0.63-0.69

excluding guideline misinterpretation, it was in 68% of cases. For the remaining cases, a panel consensus was achieved for cause and mechanism of death. Overall kappa coefficient for main cause of death for multiple observers and multiple test results was 0.81 (95% C.I. 0.80-0.83) and after excluding guideline misinterpretations, it was 0.86 (95% C.I. 0.84-0.87). Overall kappa coefficient for subclassification of cause of death was 0.67 (95% C.I. 0.66-0.68) and after excluding guideline misinterpretation, it was 0.79 (95% C.I. 0.79-0.80). For mechanism of death, overall kappa coefficient was 0.72 (95% C.I. 0.70-0.74) and after excluding guideline misinterpretation, it was 0.78 (95% C.I. 0.76-0.79). Over each main category of cause of death and each category of mechanism, a kappa correlation coefficient with lower-upper C.I. was calculated. Table 4 shows the distribution of inter-rater agreement over these categories by the five assessors. The best agreement level for cause of death was observed for congenital anomaly. The categories placenta, prematurity/immaturity and unknown showed very good agreement. Reproducibility of the causes infection and other was fair.

DISCUSSION

We describe the development of a new classification system for cause and mechanism of perinatal mortality initiated by the audit of perinatal mortality and the problems we faced using existing systems. A pathophysiological background was the basis for this system, and our purpose was to identify the unique initial demonstrable entity on the causal pathway to death for the purpose of counselling

and prevention. We assessed the inter-rater agreement for underlying cause and mechanism of perinatal mortality and found this system to be unambiguous and reproducible.

Confusion between mechanism of death and risk factors with cause of death is a problem when classifying.[3] Morrison and Olsen et al.[30] used placental insufficiency and postmaturity as cause of death in their classification. In our system, placental insufficiency is a mechanism of death and postmaturity a contributing factor (risk factor) because these are not the first step on the causal pathway to death. Whitfield et al.[32] use intrauterine growth restriction (IUGR) as the cause of death in their classification; in our system, this would be considered a contributing factor since cause of death may differ in different cases with IUGR. In accordance to Hanzlick,[3] we defined the mechanism of death as the organ failure through which the underlying cause of death ultimately exerts its lethal effect. Fetuses or neonates dying from the same underlying cause may do so because of different mechanisms of death. In the case of a pregnant mother with pre-eclampsia, with a fetus, who died in utero due to placental insufficiency, the cause of death is placental bed pathology. In another mother with pre-eclampsia mother, who delivered by caesarean section and the child died due to respiratory insufficiency, the cause of death is also placental bed pathology. Information about the mechanism of death may be as valuable as the underlying cause of death itself, to evaluate and predict institutional needs for the care of such women. Although risk factors influence the causal pathway to death, they should not be considered as the cause of death.

If the aim of classification of death is to go back to the initial step on the causal pathway because of interest in prevention, it becomes vital that cause-of-death groups consist of pathophysiological entities and not clinical manifestations of these entities. Many classification systems consist of cause-of-death groups that encompass clinical conditions such as pre-eclampsia,[29] antepartum haemorrhage,[13] breech presentation[18] and intraventricular haemorrhage of the neonate.[21] In this respect, it does not seem appropriate to retain separate categories for deaths, with evidence of asphyxia.[6,11,14,17,21,22,32,33,42] Asphyxia is a clinical condition of an underlying cause of death and can be defined in most cases. If for other reasons, one is interested in the number of women with a perinatal death and clinical conditions such as pre-eclampsia or pre-existent hypertension, it is possible to record these as contributing factors in the Tulip classification.

Simple, short, easy to use classification systems may seem preferable.[17,23,33,38] However, the difficulty when focusing on aetiology of death if using a classification system such as the Wigglesworth classification,[33] is that it remains very general. For example, all nonmalformed stillbirths are classified in the group: unexplained death prior to the onset of labour. Nevertheless, for many stillbirths, the cause of death is evident. While the Tulip system is more complex than some, the advantages more

than outweigh the complexity in application. Systems without subclassification of main causes can be too crude as is seen in a descriptive classification of underlying cause of death by de Galan-Roosen et al.[2] This system has been validated with good reproducibility (kappa =0.7) and a low percentage (7%) of unclassifiable cases, both important requirements for a good classification. Yet, 53% of cases is classified in the group placenta pathology, 32% in the subgroup acute and 21% in the subgroup chronic, without further subclassification. We divided the group placenta into four subgroups and divided the subgroup placental pathology into three further subgroups. This subclassification may prove useful when counselling parents, since different placental pathologies differ in recurrence risk.

It should be preferable to allocate every mortality case to one cause-of-death category in a system only,[6,43] independent of the clinician and his or her speciality.[23] Clear guidelines are necessary with criteria for categorisation, definition of terms and case examples.[32] Often these are missing or stated very briefly in other systems.[6-9,15,16,19,21,22,24-26,29-31,33,34]

However, in certain cases, differences in opinion between panel members regarding allocation of underlying cause of death in our system occurred. One of these was the debate about the start of the chain of events to death regarding prematurity. Pathways to preterm delivery are multifactorial.[44] Infection is often regarded as an important factor in PPROM or preterm labour but cannot always be assigned as first step on the causal pathway to death. After debate, we considered infection as cause of death if there was clear microbiological evidence of infection with matching clinical and pathological infectious findings, concluding that the infection initiated the chain of events to death. For cases in which it is not possible to go back further in the chain of events than PPROM or preterm labour because of lack of clear evidence of an earlier step on the pathway, prematurity should be assigned as cause of death in the Tulip classification. A secondary infection will be expressed in an 'infectious' mechanism of death: *multi-organ failure* or origin of mechanism such as *sepsis*. This partly explains why our cause of death group *infection* (n=6) consists of far less deaths than our *prematurity/immaturity* group (n=95).

It is unsatisfactory to classify a high percentage of cases as unknown. In 11% of our cases, a cause of death could not be allocated. Due to differences in definition, it is difficult to compare this percentage with the percentages of "unknown" or "unclassifiable" in other studies. In one-third of these deaths, the cause remained unknown despite thorough investigation, and in two-thirds of deaths, the cause remained unknown because important information was missing. This was most often because of missing diagnostic test results, such as results of chromosomal examination (because of either failure to perform the test or failure of cultures) and microbiological or pathological investigation. This suggests that many of these deaths may be underinvestigated rather than truly unexplained and that a decrease

in the percentage of *unknown* causes can be achieved by adequate diagnostic procedures after perinatal death.

Inter-rater agreements were calculated for the assessors who originally developed the system. However, these kappas illustrate good multidisciplinary agreement. In other studies, kappa scores vary. Low scores of 0.45-0.62 were observed for the validation study of Cole's classification, 0.50-0.59 for Hey's classification and 0.50-0.68 for the "New Wiggelsworth" classification.[38] These kappa scores were for external assessors. In the study of de Galan-Roosen et al.[20] an overall kappa for main causes of death of 0.70 (95% C.I. 0.68-0.72) was calculated. The highest kappa scores of 0.85-0.90 were observed for the classification by Chan et al.[15]. Both inter-rater agreements were calculated for the original assessors who developed the system. Disagreement in our panel was partly because of failure to comply with the definitions and working rules and partly because of differences in the interpretation of the sequence to death, minimal information available or an unsatisfactory narrative. The importance of individual assessors following guidance is exemplified by the rise in the kappa scores for cause of death and subclassification after removal of cases where the guideline rules had been violated.

Due to increased knowledge, newly developed techniques and methods of investigation, the patterns of causes of death have changed during time.[21,37] Therefore, a classification system must be designed in such a way that future knowledge allows expansion.[4] The Tulip system allows adaptation to medical advances. To illustrate this, deaths defined as congenital anomaly, other, multiple-organ systems in the Tulip classification may be allocated as syndrome, monogenic in the future.

In conclusion, use of a large dataset of perinatally related deaths has allowed our multidisciplinary team to construct groups of cause and mechanism of death into a functional pathophysiological classification that directs attention towards initial causation and mechanism in order to focus on prevention of perinatal deaths. The unambiguous Tulip classification is a well-defined, single-cause system, with clear guidelines and case examples. The Tulip gives a good multidisciplinary inter-rater agreement, with a low percentage of unknown causes and is easily applied by a team of clinicians when Tulip guidelines are followed. The classification is currently in use in the Netherlands for national audit studies.

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Appendix. Case examples

Example 1	
Mother: 40 years of age, G3P1A1, born at 20 weeks of gestation, girl, 260 grams, termination of pregnancy with prostaglandines	
cause of death:	congenital anomaly; chromosomal defect; numerical: trisomy 13 (1.1.1)
mechanism:	placental insufficiency (5)
origin of mechanism:	induction
contributing factor:	none
co-morbidity:	psoriasis
Example 2	
Mother: 38 years of age, G2P1, 29 weeks of gestation, boy, 1500 grams, died in utero	
cause of death:	placental bed pathology (2.1.0)
mechanism:	placental insufficiency (5)
origin of mechanism:	placental infarction
contributing factor:	pre-existing hypertension, factor II mutation
co-morbidity:	none
Example 3	
Mother: 27 years of age, G2P0, born at 26 weeks of gestation, girl, 505 grams, died 8 weeks after birth	
cause of death:	placental bed pathology (2.1.0)
mechanism:	respiratory insufficiency (3)
origin of mechanism:	chronic lung disease
contributing factor:	pre-eclampsia with antihypertensive treatment, hyperhomocysteinemia, smoking, IUGR, prematurity
co-morbidity:	alfa-thalassaemie
Example 4	
Mother: 22 years of age, G2P1, 26 weeks of gestation, boy, 835 grams, died during labour	
cause of death:	prematurity; PPROM (3.1.0)
mechanism:	cardiocirculatory insufficiency (1)
origin of mechanism:	umbilical cord occlusion
contributing factor:	breech presentation, chorioamnionitis, small placental infarction
co-morbidity:	none
Example 5	
Mother: 35 years of age, G4P3, 37 weeks of gestation, boy, 3430 grams, died in utero	
cause of death:	infection ascending (4.2)
mechanism:	multi-organ failure (2)
origin of mechanism:	intrauterine infection
contributing factor:	none
co-morbidity:	asthma
Example 6	
Mother: 29 years of age, G2P0, 35 weeks of gestation, boy, 2490 grams, died in utero	
cause of death:	other; maternal disease, diabetes mellitus type I (5.2)
mechanism:	cardio-circulatory insufficiency (1)
origin of mechanism:	ketoacidosis
contributing factor:	language/culture barrier
co-morbidity:	hernia nuclei pulposi

REFERENCES

1. Buitendijk S, Zeitlin J, Cuttini M, Langhoff-Roos J, Bottu J. Indicators of fetal and infant health outcomes. Eur J Obstet Gynecol Reprod Biol. 2003;111 Suppl 1:S66-S77.
2. Galan-Roosen AE, Kuijpers JC, van der Straaten PJ, Merkus JM. Evaluation of 239 cases of perinatal death using a fundamental classification system. Eur J Obstet Gynecol Reprod Biol. 2002;103:37-42.
3. Hanzlick R. Principles for including or excluding 'mechanisms' of death when writing cause-of-death statements. Arch Pathol Lab Med. 1997;121:377-380.
4. Kramer MS, Liu S, Luo Z, Yuan H, Platt RW, Joseph KS. Analysis of perinatal mortality and its components: time for a change? Am J Epidemiol. 2002;156:493-497.
5. Wigglesworth JS. Causes and classification of fetal and perinatal death. Fetal and perinatal pathology. London: Blackwell science;1998:75-86.
6. Alberman E, Botting B, Blatchley N, Twidell A. A new hierarchical classification of causes of infant deaths in England and Wales. Arch Dis Child. 1994;70:403-409.
7. Alberman E, Blatchley N, Botting B, Schuman J, Dunn A. Medical causes on stillbirth certificates in England and Wales: distribution and results of hierarchical classifications tested by the Office for National Statistics. Br J Obstet Gynaecol. 1997;104:1043-1049.
8. Alessandri LM, Chambers HM, Blair EM, Read AW. Perinatal and postneonatal mortality among Indigenous and non-Indigenous infants born in Western Australia, 1980-1998. Med J Aust. 2001;175:185-189.
9. Autio-Harmainen H, Rapola J, Hoppu K, Osterlund K. Causes of neonatal deaths in a pediatric hospital neonatal unit. An autopsy study of a ten-year period. Acta Paediatr Scand. 1983;72:333-337.
10. Baird D, Walker J, Thomson AM. The causes and prevention of stillbirths and first week deaths. III. A classification of deaths by clinical cause; the effect of age, parity and length of gestation on death rates by cause. J Obstet Gynaecol Br Emp. 1954;61:433-448.
11. Bound JP, Butler NR, Spector WG. Classification and causes of perinatal mortality. Br Med J. 1956;12:1191-1196.
12. Bound JP, Butler NR, Spector WG. Classification and causes of perinatal mortality. II. Br Med J. 1956;44:1260-1265.
13. Butler NR, Alberman ED. Perinatal problems: the second report of the 1958 British Perinatal Mortality Survey. 1969. Edinburgh, E & S Livingstone LtD.
14. Butler N.R, Bonham DG. Perinatal mortality: the first report of the 1958 British Perinatal Mortality Survey. 1963. Edinburgh, E & S Livingstone Ltd.
15. Chan A, King JF, Flenady V, Haslam RH, Tudehope DI. Classification of perinatal deaths: development of the Australian and New Zealand classifications. J Paediatr Child Health. 2004;40:340-347.
16. Chang A, Keeping JD, Morrison J, Esler EJ. Perinatal death: audit and classification. Aust N Z J Obstet Gynaecol. 1979;19:207-211.
17. Cole S, Hartford RB, Bergsjo P, McCarthy B. International collaborative effort (ICE) on birth weight, plurality, perinatal, and infant mortality. III: A method of grouping underlying causes of infant death to aid international comparisons. Acta Obstet Gynecol Scand. 1989;68:113-117.

18. Cole SK, Hey EN, Thomson AM. Classifying perinatal death: an obstetric approach. Br J Obstet Gynaecol. 1986;93:1204-1212.

19. Fairweather DV, Russell JK, Anderson GS, Bird T, Millar DG, Pearcy PA. Perinatal mortality in Newcastle upon Tyne 1960-62. Lancet. 1966;1:140-142.

20. Galan-Roosen AE, Kuijpers JC, van der Straaten PJ, Merkus JM. Fundamental classification of perinatal death. Validation of a new classification system of perinatal death. Eur J Obstet Gynecol Reprod Biol. 2002;103:30-36.

21. Hey EN, Lloyd DJ, Wigglesworth JS. Classifying perinatal death: fetal and neonatal factors. Br J Obstet Gynaecol. 1986;93:1213-1223.

22. Hovatta O, Lipasti A, Rapola J, Karjalainen O. Causes of stillbirth: a clinicopathological study of 243 patients. Br J Obstet Gynaecol. 1983;90:691-696.

23. Keeling JW, MacGillivray I, Golding J, Wigglesworth J, Berry J, Dunn PM. Classification of perinatal death. Arch Dis Child. 1989;64:1345-1351.

24. Knutzen VK, Baillie P, Malan AF. Clinical classification of perinatal deaths. S Afr Med J. 1975;49:1434-1436.

25. Lammer EJ, Brown LE, Anderka MT, Guyer B. Classification and analysis of fetal deaths in Massachusetts. JAMA. 1989;261:1757-1762.

26. Langhoff-Roos J, Borch-Christensen H, Larsen S, Lindberg B, Wennergren M. Potentially avoidable perinatal deaths in Denmark and Sweden 1991. Acta Obstet Gynecol Scand. 1996;75:820-825.

27. Low JA, Boston RW, Cervenko FW. A clinical classification of the mechanisms of perinatal wastage. Can Med Assoc J. 1970;102:365-368.

28. Low JA, Boston RW, Crussi FG. Classification of perinatal mortality. Can Med Assoc J. 1971;105:1044-1046.

29. McIlwaine GM, Howat RC, Dunn F, Macnaughton MC. The Scottish perinatal mortality survey. Br Med J. 1979;2:1103-1106.

30. Morrison I, Olsen J. Weight-specific stillbirths and associated causes of death: an analysis of 765 stillbirths. Am J Obstet Gynecol. 1985;152:975-980.

31. Naeye RL. Causes of perinatal mortality in the U.S. Collaborative Perinatal Project. J Am Med Assoc. 1977;238:228-229.

32. Whitfield CR, Smith NC, Cockburn F, Gibson AA. Perinatally related wastage--a proposed classification of primary obstetric factors. Br J Obstet Gynaecol. 1986;93:694-703.

33. Wigglesworth JS. Monitoring perinatal mortality. A pathophysiological approach. Lancet. 1980;2:684-686.

34. Winbo IG, Serenius FH, Dahlquist GG, Kallen BA. NICE, a new cause of death classification for stillbirths and neonatal deaths. Neonatal and Intrauterine Death Classification according to Etiology. Int J Epidemiol. 1998;27:499-504.

35. Campbell MK, Webster KM. Age at neonatal death in Ontario, 1979-1987: implications for the interpretation of mortality markers. Paediatr Perinat Epidemiol. 1993;7:426-433.

36. Cartlidge PH, Stewart JH. Effect of changing the stillbirth definition on evaluation of perinatal mortality rates. Lancet. 1995;346:486-488.

37. Fretts RC, Boyd ME, Usher RH, Usher HA. The changing pattern of fetal death, 1961-1988. Obstet Gynecol. 1992;79:35-39.

38. Settatree RS, Watkinson M. Classifying perinatal death: experience from a regional survey. Br J Obstet Gynaecol. 1993;100:110-121.

39. Elstein AS, Schwarz A. Clinical problem solving and diagnostic decision making: selective review of the cognitive literature. BMJ. 2002;324:729-732.

40. Pearson GA, Stickley J, Shann F. Calibration of the paediatric index of mortality in UK paediatric intensive care units. Arch Dis Child. 2001;84:125-128.

41. Fleiss JL. Statistical methods for rates and proprtions. 2nd edition. 1981. New York, Wiley.

42. Winbo IG, Serenius FH, Dahlquist GG, Kallen BA. A computer-based method for cause of death classification in stillbirths and neonatal deaths. Int J Epidemiol. 1997;26:1298-1306.

43. Elamin S, Langhoff-Roos J, Boedker B, Ibrahim SA, Ashmeig AL, Lindmark G. Classification of perinatal death in a developing country. Int J Gynaecol Obstet. 2003;80:327-333.

44. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000;342:1500-1507.

Chapter 2

A placental cause of intrauterine fetal death depends on the perinatal mortality classification system used

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ABSTRACT

Different classification systems for the cause of intrauterine fetal death (IUFD) are used internationally. About two thirds of these deaths are reported as unexplained and placental causes are often not addressed. Differences between systems could have consequences for the validity of vital statistics, for targeting preventive strategies and for counselling parents on recurrence risks. Our objective was to compare use of the Tulip classification with other currently used classification systems for causes of IUFD. We selected the extended Wigglesworth classification, modified Aberdeen and the classifications by Hey, Hovatta, de Galan-Roosen and Morrison. We also selected the ReCoDe system for relevant conditions, comparable to contributing factors in the Tulip classification. Assessors performed panel classification for 485 IUFD cases in the different systems after individual investigation of structured patient information. Distribution of cases into cause of death groups for the different systems varied, most of all for the placental and unknown groups. Systems with a high percentage of cases with an unknown cause of death and death groups consisting of clinical manifestations only are not discriminatory. Our largest cause of death group was placental pathology and classification systems without placental cause of death groups or minimal subdivision of this group are not useful in modern perinatal audit as loss of information occurs. The most frequent contributing factor was growth restriction. This illustrates the vital role of the placenta in determination of optimal fetal development. In the Tulip classification, mother, fetus and placenta are addressed together. The system has a clear defined subclassification of the placenta group, a low percentage of unknown causes and is easily applied by a multidisciplinary team. A useful classification aids future research into placental causes of IUFD.

INTRODUCTION

There are intensified demands on medical, political and epidemiological grounds for proper determination and classification of cause of perinatal death.[1-5] The largest subgroup of perinatal mortality worldwide is the stillbirth group consisting of intrauterine fetal deaths (IUFD) and intrapartum deaths. Current use of classification systems for analyses of this subgroup consistently report of about two thirds of these deaths as being unexplained.[6] Classification of cause of death is needed for the individual patient in the process of mourning, for the purpose of counselling and prevention and for comparison of health care nationally and internationally. Classification of IUFD is complex due to the complicated pathophysiological processes encountered in the mother, fetus and placenta, and as a result of their interaction.[7] The multiplicity of contributing factors and the different background of the clinicians involved, adds to the complexity.

Different classification systems have been designed for different reasons with different approaches, definitions, levels of complexity and availability of guidelines. No single system is universally accepted and each has strengths and weaknesses.[8,9] Problems occur during use and comparison of different systems. Our research group developed a new classification system for perinatal mortality: the Tulip classification, in anticipation of current needs.[8] This system was designed by a multidisciplinary panel. Placental causes of death formed our largest cause of death group. This is in accordance with others who also found placental causes of death in up to 60% of perinatal mortality cases.[2,10-13] However, availability of a placental death group varies in internationally used classification systems.

Our goal for this study was to investigate underlying cause of death for an IUFD group after evaluation of clinical and diagnostic information. Special interest was in placental causes. Our objective was to compare use of the Tulip classification with other currently used classification systems for IUFD. Question was whether information is gained or lost by classification in the different systems. This could have consequences for counselling parents on recurrence risks, for targeting placental research and preventive strategies, and for the validity of vital statistics.

MATERIALS AND METHODS

In 2002 we initiated a national study on IUFD at the University Medical Centre in Groningen (UMCG) with 50 participating hospitals throughout the Netherlands. Inclusion criteria for the study were singleton IUFD's diagnosed antepartum after 20 weeks of gestation. For each included IUFD a case record form was filled in and a standard diagnostic workup protocol was performed.

Patient information sets included baseline characteristics such as date of delivery, gestational age, medical and obstetric history; maternal characteristics; fetal characteristics including fetal and placental weights at birth; pregnancy details and obstetric discharge letters. Apart from these characteristics, diagnostic test results were available including: pathological findings concerning autopsy and placental investigation; maternal blood tests; maternal viral serology; fetal blood tests; fetal viral serology; cultures from mother, fetus and placenta; and chromosomal investigation. Autopsy and placental examination were performed by local pathologists in participating hospitals after parental consent was obtained. No national pathological guidelines regarding autopsy and placental examination after IUFD exist, therefore we urged participating pathologists to follow our study guidelines for autopsy and placental examination based on the guidelines published by the Royal College of Obstetricians and Gynaecologists[14] and the Royal College of Pathologists and the College of American Pathologists.[15,16]
After patient sets were made as complete as possible panel classification sessions were initiated. Procedures were agreed upon in advance. For fetal and placental weights at birth gestational age at determination of IUFD was used. Small for gestational age (SGA) was defined as birth weight < 10^{th} percentile.[17] Placenta hypoplasia was defined as an absolute too low placenta weight < 10^{th} percentile and/or a too low placenta/birth weight ratio.[18] We defined placental bed pathology for preterm cases as any infarctions found at placental histology and for term cases as extensive infarction that affected > 10% of the placental area.[19] Cause of death "placental bed pathology" was allocated if in our opinion the percentage of infarcted parenchyma in relation to the weight of the placenta was severe enough to cause death. The classification panel consisted of two obstetricians, an obstetric resident, and a paediatric pathologist. All panel members prepared each case individually using the patient information sets where after panel discussions were held and a panel consensus on cause of death was agreed upon. No other information sources were consulted. Only one underlying cause of death could be allocated. For each classification system we added "problematic classification" as cause of death group.. This cause was classified if allocation of cause of death caused confusion for a system and/or two cause of death groups could be allocated at the same time.

Used classification systems for cause of death

After panel discussion on the basis of use of existing classifications and current obstetric, pathologic and genetic literature on causes of IUFD we selected six classification systems besides the Tulip classification. These systems represent different approaches of classification with different definitions. The selected systems were as follows: the Extended Wigglesworth,[20] the Modified Aberdeen,[21]

classification by Hey et al.,[22] by Hovatta et al.,[23] by Galan-Roosen et al.[24] and by Morrison and Olsen.[25] The reason for choice of the system as well as the system itself will be discussed in the following paragraphs.

The *Tulip* classification is a single cause classification system aiming to identify the initial demonstrable pathophysiological entity initiating the chain of events that has irreversibly led to death. Cause of death is based on the combination of clinical findings and diagnostic test results, including pathological findings for the purpose of counselling and prevention.[8] As our goal was to particularly focus on placental causes of death we discuss this part of the guideline.

Placental cause of death. Cause of death is explained by a placental pathological abnormality supported by the clinical findings.

(2.1) *Placental bed pathology.* Inadequate spiral artery remodelling and/or spiral artery pathology leading to uteroplacental vascular insufficiency such as placental infarction and abruption.

(2.2) *Placental pathology.* Placental pathology originated during development of the placenta itself, abnormalities in the parenchyma or localisation of the placenta. (2.2.1) *Development.* Morphologic abnormalities arise because of abnormal developmental processes. Examples: placenta circumvallata, vasa praevia, villus immaturity, and placenta hypoplasia. (2.2.2) *Parenchyma.* Acquired placenta parenchyma disorders of the villi or intervillous space. Examples: fetal thrombotic vasculopathy, maternal floor infarct, villitis of unknown origin, massive perivillous fibrin deposition and fetomaternal haemorrhage without obvious cause. (2.2.3) *Abnormal localisation.* Example: placenta praevia.

(2.3) *Umbilical cord complication.* Example: true knot with occlusion of the umbilical vessels.

(2.4*) Not otherwise specified.* The cause of death can be allocated to the group placenta but, because of the combination of different placenta subclassifications, a choice cannot be made as to what was first in the chain of events leading to death".

The *extended Wigglesworth* classification, the *modified Aberdeen* and the classification by *Hey et al.*[20-22] are based on the earliest developed classification systems. These systems have different approaches and are the most commonly used systems for British statistics.[3] In addition, the extended Wigglesworth and the modified Aberdeen[20,21] are most widely used throughout the world.[26-31] Wigglesworth advocated a pathophysiological approach and the goal of the classification is to subdivide cases into groups with clear implications for priorities for prevention and alterations in clinical management. The modified Aberdeen is a clinicopathological classification, the first version was proposed by Baird et al.[21] and aim is to classify each death in accordance with the factor which probably

initiated "the train of events ending in death". It is almost entirely based on clinical information as in the experience of the designers of the system post-mortem examinations fail to explain cause of death in many cases. The classification by Hey et al.[22] is based on the Bound classification.[32,33] This classification has a pathologic approach based on fetal and neonatal entities and aim is to define the clinicopathological process within the baby and the way they contribute to, and help to explain the baby's death. *Hovatta et al.*[23] designed a system especially for the group of stillbirths. Aim is to classify underlying cause of death considering both clinical and autopsy findings. The classification groups are based on maternal, fetal, placental or a combination of these entities. Definitions for the placental causes, however, do not exist.

The classification by *Galan-Roosen et al.* is one of the few systems based on maternal, fetal and placental entities.[24] Aim is to serve prevention and classify underlying cause of death with a clinicopathological approach based on the entities that initiated the chain of events leading to death. The group placenta pathology is defined as follows in the guideline.

(1) *Acute/subacute placental pathology:* total or partial abruption of the placenta, placental haematomata with intervillous thrombosis, marginal haemorrhage, subchorial haematoma, placental infarction >10%, velamentous insertion with vasolaceration or compression of the cord, and cord prolapse/compromise. Sometimes no placenta pathology can be found. Clinical manifestations in the fetus are signs of asphyxia with (in the subacute fase) time to aspirate meconium-stained amniotic fluid.

(2) Chronic/progressive placental pathology: placental maldevelopment like in placenta praevia, uterine malformation or septum. Maternal circulation disorders and terminal villus deficiency like in pregnancy-induced hypertension (PIH), pre-eclampsia, and thrombophilia. Also when coagulation disorders are found in blood samples of the mother like in systemic lupus erythematosus (SLE). Examples: massive perivillous fibrin depositions, villitis of unknown origin, and diabetic changes in the placenta: pale, large and immature villi with oedema. Clinical manifestations of chronic placenta pathology in the fetus can be signs of small for gestational age.

The classification by *Morrison and Olsen*[25] is especially designed for stillbirths based on the clinicopathological classification of the British perinatal mortality survey.[34,35] The major contributing cause of death selected is based on maternal entities with an obstetric clinical approach and divided into specific weight categories. Aim is to serve prevention and study or define implications for that geographical area or clinic studied. Their group *hypoxia: placental insufficiency* is defined as: "autopsy evidence of hypoxia with appropriate weight for gestation, with meconium or meconium-stained membranes in vertex presentation; or

birth weight/placental weight ratio > 7:1 or placental infarcts > 25%. The group *hypoxia; cord accidents/compression* is defined as: nuchal cord ≥ 2, or true knot, or prolapse, or perforation at amniocentesis".

Relevant conditions

The latest published classification is the system by Gardosi et al. in 2005.[3] Their ReCoDe classification seeks to establish relevant conditions at death taking into account mother, fetus and placenta. This system is not designed for allocation of cause of death. From the start of our panel sessions, we classified contributing factors for the Tulip classification besides cause of death. Our contributing factors are defined as other known factors on the causal pathway to death e.g. risk factors. These contributing factors are very similar to ReCoDe's relevant conditions. Combining information from our Tulip causes of death and contributing factors it was therefore possible to classify relevant conditions according to the ReCoDe classification.

RESULTS

During the four year period of 2002-2006 we included 485 IUFD's. Median gestational age was 31 weeks and 4 days (range 20-42 weeks 1 day). Median age of the mother was 30 years (range 18- 46 years). Of the 485 IUFD's 263 were boys, 221 girls and for one case sex at birth could not be determined and no information on chromosomal or pathological examination was available. Autopsy was performed in 348 (71.7%) cases and external macroscopic fetal examination by a pathologist without autopsy in 18 cases (3.7%). Placental examination was performed in 481 cases (99.1%). The extent to which the placental examination guidelines were followed differed between cases.

During the panel sessions all IUFD's were classified according to the eight selected classification systems. For the *Tulip* classification distribution of causes of death is shown in Table 1. Largest cause of death group for 312 cases was placenta (64.3%). Largest placenta subgroups were placental bed pathology in 166 cases (34.2%) and placental pathology; development in 76 cases (15.7%). No cases were allocated to the group prematurity as we studied an IUFD cohort. Eight cases were allocated to the infection group. In 113 cases (23.3%) cause of death remained unknown, and in 30 cases important information was missing.

Distribution of causes of death for the *extended Wigglesworth* the *modified Aberdeen,* the classification by *Hey et al.,* by *Hovatta et al.,* by *Galan-Roosen et al.* and by *Morrison and Olsen* are shown in Tables 2-7, respectively. Relevant

Table 1. Tulip classification: causes.

Cause of death	n (% of total)		Subclassification			n
1 Congenital anomaly	28	1	Chromosomal defect	1	Numerical	12
	(5.8)			2	Structural	2
				3	Microdeletion/ uniparental disomy	-
		2	Syndrome	1	Monogenic	-
				2	Other	2
		3	Central nervous system			-
		4	Heart and circulatory system			3
		5	Respiratory system			-
		6	Digestive system			1
		7	Urogenital system			-
		8	Musculoskeletal system			-
		9	Endocrine/metabolic system			-
		10	Neoplasm			3
		11	Other	1	Single organ	-
				2	Multiple organ	5
2 Placenta	312	1	Placental bed pathology			166
	(64.3)	2	Placental pathology	1	Development	76
				2	Parenchyma	16
				3	Localisation	-
		3	Umbilical cord complication			25
		4	Not otherwise specified			29
3 Prematurity/ Immaturity	-	1	PPROM			-
		2	Preterm labour			-
		3	Cervical incompetence			-
		4	Iatrogenous			-
		5	Not otherwise specified			-
4 Infection	8	1	Transplacental			5
	(1.6)	2	Ascending			3
		3	Neonatal			-
		4	Not otherwise specified			-
5 Other	24	1	Fetal hydrops of unknown origin			16
	(4.9)	2	Maternal disease			8
		3	Trauma	1	Maternal	-
				2	Fetal	-
		4	Out of the ordinary			-
6 Unknown	113	1	Despite thorough investigation			83
	(23.3)	2	Important information missing			30
Total	100					485

Table 2. Extended Wigglesworth: causes

Code	Classification	%	Subclassification	n
1.0	Congenital defect/malformation	6.0		29
2.0	Unexplained antepartum fetal death	88.5		429
3.0	Death from intrapartum asphyxia, anoxia or trauma	-		-
4.0	Immaturity	-		-
5.0	Infection	1.6		8
6.1	Due to other specific causes	3.7	Fetal conditions	18
6.2			Neonatal conditions	-
6.3			Paediatric conditions	-
7.0	Due to accident or non-intrapartum trauma	-		-
8.0	Sudden infant deaths, cause unknown	-		-
9.0	Unclassifiable	0.2		1
10.0	Problematic classification	-		-
Total		100		485

Table 3. Modified Aberdeen: causes

Code	Classification	%	Subclassification	n
1	Congenital anomaly	6.6	Neural tube defects	2
2			Other anomalies	30
3	Isoimmunisation	-	Due to Rhesus (D) antigen	-
4			Due to other antigens	-
5	Pre-eclampsia	6.4	Pre-eclampsia without APH	28
6			Pre-eclampsia complicated by APH	3
7	Antepartum haemorrhage (APH)	9.3	With placenta praevia	1
8			With placental abruption	38
9			Of uncertain origin	6
10	Mechanical	4.1	Cord prolapse or compression with vertex or face presentation	18
11			Other vertex or face presentation	-
12			Breech presentation	-
13			Oblique or compound presentation, uterine rupture etc.	2
14	Maternal disorder	8.7	Maternal hypertensive disease	10
15			Other maternal disease	24
16			Maternal infection	8
17	Miscellaneous	3.7	Neonatal infection	-
18			Other neonatal disease	-
19			Specific fetal conditions	18
20	Unexplained	60.4	Equal or greater than 2.5 kg	90
21			Less than 2.5 kg	203
22	Unclassified	-	Unclassifiable	-
23	Problematic classification	0.8		4
Total		100		485

conditions for our 485 cases according to the *ReCoDe* classification by Gardosi et al. are shown in table 8.

The extended Wigglesworth and the modified Aberdeen, which are amongst the internationally most used classification systems have an excessive number of unexplained cases and do not include placental causes of death in their system (Table 9). The Tulip system illustrates that a large group of these unexplained deaths have a placental cause of death. For the modified Aberdeen 293 cases were "unexplained" and four cases were "problematic". Contrary, eight "unknown" cases in the Tulip classification were allocated a known cause in the modified Aberdeen: congenital anomaly (n=1); pre-eclampsia (n=1); antepartum haemorrhage (n=2) and maternal disorder (n=4). For the extended Wigglesworth classification 429 cases were unexplained and one case was problematic, and one case classified

Table 4. Classification by Hey et al: causes

Code	Classification	%	Subclassification	n
1	Congenital anomaly	6.0	Chromosomal defect	13
2			Inborn error of metabolism	-
3			Neural tube defect	1
4			Congenital heart defect	3
5			Renal abnormality	-
6			Other malformation	12
7	Isoimmunisation			-
8	Asphyxia	88.5	Antepartum	429
9			Intrapartum	-
10	Birth trauma			-
11	Pulmonary immaturity			-
12	Hyaline membrane disease			-
13			With IVH	-
14			With infection	-
15	Intracranial haemorrhage		Intraventricular haemorrhage	-
16			Other intracranial bleeding	-
17	Infection	1.9	Necrotising enterocolitis	-
18			Antepartum	9
19			Intrapartum	-
20			Postpartum	-
21	Miscellaneous	3.7	Miscellaneous	18
22	Unclassifiable or unknown		Cot death	-
23			Unattended delivery	-
24			Other undocumented death	-
25	Problematic classification			-
Total				485

Table 5. Classification by Hovatta et al: causes

Code	Classification	%	Subclassification	n
1.0	Abruption of the placenta	7.8		38
2.0	Large placental infarction	21.9		106
3.0	Cord complication	5.2		25
4.1	Other placental feature	27.2	Severe pre-eclampsia	5
4.2			Cholestasis of pregnancy	1
4.3			Twin pregnancy	-
4.4			Immature birth	-
4.5			Severe maternal trauma	-
4.6			Uterine anomaly	-
4.7			Other causes	126
5.0	Asphyxia for unexplained reasons	8.2		40
6.0	Maternal isoimmunization	-		-
7.1	Fetal bleeding	1.2	Fetofetal transfusion	-
7.2			Fetomaternal transfusion	5
7.3			Other bleeding	1
8.0	Severe chorioamnionitis	1.0		5
9.0	Major malformations	5.8		28
10.0	Unexplained	19.4		94
11.0	Problematic classification	2.3		11
Total		100		485

as “unknown” in the Tulip classification was classified as congenital defect/ malformation in the Wigglesworth.

The largest group in the Tulip classification consisted of placental causes: 312 cases (64.3%). We plotted the Tulip placental causes against the causes of death in classification systems with at least one placental cause of death category.[23-25] The classifications by Hovatta et al., Galan-Roosen et al. and Morrison and Olsen have fewer unexplained cases than the other used systems. These systems contain placental causes of death but as illustrated in Table 10 there is minimal subclassification of these categories. Besides, some causes of death groups represent clinical conditions that raise confusion.

DISCUSSION

In anticipation of audit purposes and further international comparison of causes we investigated different classification systems for cause of IUFD. Our focus was on placental causes of death as these are becoming more and more recognized. We describe comparison of eight classification systems. The Tulip classification

Table 6. Classification by de Galan-Roosen et al: causes

Code	Classification	%	Subclassification	Specification	n
1.1.0	Trauma	-	Antenatal		-
1.2.0			At birth		-
1.3.0			Postnatal		-
2.1.1	Infection	1.6	Antenatal	Haematogenous	5
2.1.2				Transamniotic	3
2.2.0			Postnatal		-
3.1.0	Placenta/cord pathology	44.5	Acute/subacute		98
3.2.0			Chronic/progressive		118
4.1.0	Maternal immune system pathology	-	Blood type incompatibility		-
4.2.0			Blood platelet antibody		-
5.1.0	Congenital malformations incompatible with life	4.9	Hereditary		-
5.2.0			Non-hereditary		24
6.1.0	Prematurity/immaturity complications	-	Cervix incompetence		-
6.2.0			Preterm labour iatrogenous		-
6.3.0			Preterm labour eci		-
7.1.0	Unclassifiable	26.6	Despite thorough examination		99
7.2.0			Important information missing		30
8.0.0	Problematic classification	22.3			108
Total		100			485

Table 7. Classification by Morrison and Olsen: causes

Code	Classification	%	Subclassification	n
1.1	Hypoxia	55.7	Intrauterine growth retardation	121
1.2			Cord accidents/compression	25
1.3			Maternal hypertension	11
1.4			Placental insufficiency	103
1.5			Postmaturity	-
1.6			Other	10
2.1	Antepartum haemorrhage	9.1	Major abruptio placentae	41
2.2			Placenta praevia	-
2.3			Significant unexplained antepartum haemorrhage	3
3.0	Congenital anomalies	6.0		29
4.1	Diabetes	2.9	Insulin dependent	7
4.2			Gestational	7
5.0	Miscellaneous	6.0		29
6.0	Trauma	-		-
7.0	Unclassified	19.2		93
8.0	Problematic classification	1.2		6
Total		100		485

Table 8. ReCoDe: relevant conditions

Code	Classification	%	Subclassification	n
A1	Fetus	53.0	Lethal congenital anomaly	28
A2			Infection	19
A3			Non-immune hydrops	19
A4			Isoimmunisation	-
A5			Fetomaternal haemorrhage	44
A6			Twin-twin transfusion	-
A7			Fetal growth restriction	147
B1	Umbilical cord	5.6	Prolapse	-
B2			Constricting loop or knot	6
B3			Velamentous insertion	6
B4			Other	15
C1	Placenta	26.4	Abruptio	30
C2			Praevia	-
C3			Vasa praevia	-
C4			Other "placental insufficiency"	98
C5			Other	-
D1	Amniotic fluid	-	Chorioamnionitis	-
D2			Oligohydramnios	-
D3			Polyhydramnios	-
D4			Other	-
E1	Uterus	-	Rupture	-
E2			Uterine anomalies	-
E3			Other	-
F1	Mother	0.8	Diabetes	2
F2			Thyroid diseases	-
F3			Essential hypertension	-
F4			Hypertensive disease in pregnancy	-
F5			Lupus or antiphospholipid syndrome	2
F6			Cholestasis	-
F7			Drug misuse	-
F8			Other	-
G1	Intrapartum	-	Asphyxia	-
G2			Birth trauma	-
H1	Trauma	-	External	-
H2			Iatrogenic	-
I1	Unclassified	14.2	No relevant condition identified	50
I2			No information available	19
Total		100		485

Table 9. Modified Aberdeen unexplained (n=293) and problematic (n=4) and extended Wigglesworth unexplained (n=429) and problematic (n=1) versus the Tulip classification

Tulip cause of death	Placenta: placental bed pathology	Placenta: placental pathology; development	Placenta: placental pathology; parenchyma	Placenta: umbilical cord complication	Placenta: not otherwise specified	Other: fetal hydrops of unknown origin	Other: maternal disease; diabetus mellitus	Other: maternal disease; other	Unknown: despite thorough investigation	Unknown: important information missing	Total
Modified Aberdeen											
Unexplained ≥ than 2.5 kg	12	35	5		13				15	10	90
%	14	40	6		15				15	10	100
Unexplained < than 2.5 kg	75	26	7	5	12				60	18	203
%	37	13	3	2	6				30	9	100
Problematic classification	1		2							1	4
%	25		50							25	100
Extended Wiggelsworth											
Unexplained antepartum fetal death	166	75	13	25	29	2	2	5	82	30	429
%	39	17	3	6	7	0.5	0.5	1	19	7	100
Unclassifiable			1								1
%			100								100

has an extensive subdivision of the placental group, a high percentage of cases with a "known" cause of death and cause of death groups do not consist of clinical manifestations of pathophysiological entities. In the other described systems, we encountered problems concerning at least one of these items resulting in loss of specific information.

The pathophysiology of IUFD is complex and involves maternal, fetal as well as placental entities. In order to assign a cause of death these entities should be addressed together. The main focus of this study was on placental causes of death. Four of the seven classification systems we used have a placental cause of death group.[8,23-25] In these systems except for the classification by Morrison et al. a placental cause of death was the largest death group varying from 44.5% for de Galan-Roosen et al. to 64.3% in the Tulip classification. This is in accordance

with our previous study [8] and earlier published data.[2,10-13] A great number of cases classified as "unknown" in the extended Wigglesworth and the modified Aberdeen were allocated a placental cause of death in the Tulip classification (Table 9).
Minimal subclassification of placental causes results in loss of specific information, non-specific counselling of parents on recurrence risks and hampers targeting adequate preventive strategies. In this respect, the classifications by Hovatta et al., de Galan-Roosen et al. and Morrison and Olsen[23-25] seem unsatisfactory (Table 10). Use of placental subgroups triggers the discussion on definitions of these groups. Largest placental subgroup for the Tulip classification was "placental bed pathology" (n=166, 34.2%), in 42 cases this cause of death was allocated due to an abruptio placentae, in 122 cases due to placental infarctions and in 2 cases both were present. Others also worked with the same cut-off point for infarctions.[24,36] Morrison and Olsen have a higher (25%) cut-off point.[25] Second largest placenta subgroup was "placental pathology; development" in 76 cases (15.7%). In 50 cases this cause of death manifested as placental hypoplasia. We assume that part of this group comprehends cases with "placental bed pathology" as cause due to sampling error.[37] Moreover, dependent on the references used for placental weight and placenta/birth weight ratios, allocation of placental hypoplasia can vary.[18,38] To improve validity of statistics, uniformity of definitions of these large placental subgroups are needed.
The classification by de Galan-Roosen et al. has been validated with a low percentage (7%) of unclassifiable cases.[2] However, several placental pathological entities are crudely divided into two groups only. Ninety-eight cases (20.2%) were allocated to "placenta/cord pathology; acute/subacute" and 118 (24.3%) cases to "placenta/cord pathology; chronic/progressive".The second problem we faced was the large group allocated to "problematic classification" (108 cases). This was mainly due to the cases with > 10% placental infarctions (death group: "placenta/cord pathology acute/subacute") together with a small for gestational age fetus ("placenta/cord pathology chronic/progressive"). Although cause and mode of death are relevant aspects of the pathophysiology of IUFD, these items are two separate entities which should not be merged into one.
Any classification system that results in a low proportion of cases with a known cause of death does not seem to be fulfilling its purpose. Due to differences in definition, it is difficult to compare the percentages of unexplained cases in the different systems. For the total percentage of unknown cause of death groups we studied the groups "unknown", "unexplained", "unclassifiable" and "problematic classification" together. The cause of death group "unknown" varied from 0% in the classification by Hey et al. to 88.7% in the extended Wigglesworth. A short classification system such as the extended Wigglesworth may seem preferable but remains too general. This system only has cause of death groups for

Table 10. De Galan-Roosen, Hovatta and Morrison and Olsen classifications versus the Tulip classification: placental causes (n=312).

	Tulip placental cause, n= 312	
	Placental bed pathology	**Placental pathology; development**
De Galan-Roosen et al		
Placenta/cord pathology: acute/subacute	66	2
Placenta/cord pathology: chronic/progressive	14	74
Problematic classification	86	
Total	**166**	**76**
Hovatta et al		
Abruption of the placenta	38	
Large placental infarction	102	
Cord complication		
Other placental feature; severe pre-eclampsia	4	1
Other placental feature; other causes	15	74
Fetal bleeding; fetomaternal transfusion		
Fetal bleeding; other bleeding		1
Unexplained	6	
Problematic classification	1	
Total	**166**	**76**
Morrison and Olsen		
Hypoxia; intrauterine growth retardation	79	20
Hypoxia; cord accidents/compression		
Hypoxia; maternal hypertension	9	1
Hypoxia; placental insufficiency	33	43
Hypoxia; other	1	2
Antepartum hemorrhage; major abruptio placentae	41	
Antepartum hemorrhage; significant unexplained APH	1	1
Diabetes; insulin dependent		2
Diabetes; gestational		5
miscellaneous		
Problematic classification	2	2
Total	**166**	**76**

malformed stillbirths, stillbirths with clear microbiological evidence of infection or with hydrops fetalis. All other stillbirths are classified in the group: “unexplained antepartum fetal death”. Nevertheless, as is shown in Table 9 cause of death is evident for a large group of these stillbirths. For the classification by Hey et al. no deaths were classified as: “unclassifiable” or “unknown”, however 88.4% of cases were allocated to the group “asphyxia antepartum”. In our opinion asphyxia is not a cause of death but a clinical condition which is the result of an underlying cause of death and can be defined in many cases.[4] Similarly in the system of Hovatta et al

Placental pathology; parenchyma	Umbilical cord complication	Not otherwise specified	Total
3	25	1	97
10		18	116
3		10	99
16	**25**	**29**	**312**
			38
		1	103
	25		25
			5
11		25	125
5			5
			1
			6
		3	4
16	**25**	**29**	**312**
3		7	109
	25		25
			10
7		17	100
4		1	8
			41
			2
		1	3
		1	6
2			2
		2	6
16	**25**	**29**	**312**

8.3% of cases were classified as “asphyxia for unexplained reasons”. In fact these cases should be added to the cause of death group “unknown” and, therefore, their percentage of “unknown” increases from 21.6% to 29.9%. This also accounts for the group “hypoxia: intrauterine growth retardation” in the system by Morrison and Olsen (24.9%). As is shown in Table 10 most of the “asphyxia and hypoxia related” causes have placental pathology as underlying cause of death. A large group of unexplained IUFD’s is often due to design of the system itself and lack of amendment of the system to present insight into pathofysiology of IUFD. In

23.3% of cases cause remained "unknown" for the Tulip classification (Table 1). In about two thirds of deaths the cause remained unknown because important information was missing. This suggests that many of these deaths may be under investigated rather than truly unexplained. Although some systems aim to classify underlying cause of death, mechanism of death and risk factors are often mixed.[39] Cause of death groups should consist of pathophysiological entities. Many systems consist of cause of death groups that encompass clinical conditions such as pre-eclampsia,[21] antepartum haemorrhage,[25] breech presentation[21] and intraventricular haemorrhage.[22] Similarly, intrauterine growth restriction is a clinical condition of several causes of death, see Table 10.

Recently Gardosi et al.[3] published their ReCoDe classification that seeks to establish relevant conditions at death considering mother, fetus and placenta. Their system has evoked a new discussion on classification as they do not classify cause of death. The system is easy to use, as panel sessions are not needed, with retainment of important information. However, guidelines for the ReCoDe classification are less clear and this resulted in confusion of allocation of relevant conditions. Hierarchy underestimates the importance of some of the items in the lower part of the system. Results of our cohort presented in Table 8 are comparable to the stillbirth cohort presented by Gardosi et al. Largest relevant condition for our group was fetal growth restriction (30.3%) compared to 43.0%.[3] In our IUFD cohort 14.2% of cases were unclassified versus 15.2%.[3] We agree with Gardosi et al. that these relevant conditions give insight into the death. However, if classification of the underlying cause of death is added more insight is warranted. For the Tulip classification 27.6% of cases in the placental group were small for gestational age at birth versus 8.7% in the other cause of death groups illustrating diversity in cause of death for these small fetuses. Recording of growth restriction as a contributing factor is nevertheless important for management and counselling of future pregnancies.

In conclusion, comparison of seven classification systems for cause of death and one system for relevant conditions applicable for the IUFD group illustrated different problems during use. Largest cause of death group for IUFD was placental pathology, and largest contributing factor was growth restriction. This illustrates the vital role of the placenta in determining optimal fetal development. Internationally used systems without placental cause of death groups or minimal subdivision of this group are in our opinion not useful in modern perinatal audit. Systems with a low proportion of known causes of death or cause or death groups consisting of clinical manifestations of pathophysiological entities are not useful either as this results in loss of information. Of the systems we compared the Tulip classification met the requirements for a useful classification best. This classification is currently in use in the Netherlands for national audit studies.[40]

International use of the same classification system for cause of death will facilitate comparison of statistics. Future classification efforts and research should be aimed at further definition of the placental cause of death groups, investigation into the differences in clinical manifestations of placental causes of death and the prevention of these deaths.

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REFERENCES

1. Buitendijk S, Zeitlin J, Cuttini M, Langhoff-Roos J, Bottu J. Indicators of fetal and infant health outcomes. Eur J Obstet Gynecol Reprod Biol. 2003;111 Suppl 1:S66-S77.
2. Galan-Roosen AE, Kuijpers JC, van der Straaten PJ, Merkus JM. Evaluation of 239 cases of perinatal death using a fundamental classification system. Eur J Obstet Gynecol Reprod Biol. 2002;103:37-42.
3. Gardosi J, Kady SM, McGeown P, Francis A, Tonks A. Classification of stillbirth by relevant condition at death (ReCoDe): population based cohort study. BMJ. 2005;331:1113-1117.
4. Hanzlick R. Principles for including or excluding 'mechanisms' of death when writing cause-of-death statements. Arch Pathol Lab Med. 1997;121:377-380.
5. Kramer MS, Liu S, Luo Z, Yuan H, Platt RW, Joseph KS. Analysis of perinatal mortality and its components: time for a change? Am J Epidemiol. 2002;156:493-497.
6. Maternal and Child Health Consortium. CESDI 8th annual report: Confidential Enquiry of Stillbirths and Deaths during Infancy. 2001. London.
7. Wigglesworth JS. Causes and classification of fetal and perinatal death. Fetal and perinatal pathology. London: Blackwell science. 1998:75-86.
8. Korteweg FJ, Gordijn SJ, Timmer A et al. The Tulip classification of perinatal mortality: introduction and multidisciplinary inter-rater agreement. BJOG. 2006;113:393-401.
9. Silver RM, Varner MW, Reddy U et al. Work-up of stillbirth: a review of the evidence. Am J Obstet Gynecol. 2007;196:433-444.
10. Agapitos E, Papadopoulou C, Kavantzas N, Papoulias J, Antonaki V, Davaris P. The contribution of pathological examination of the placenta in the investigation of the causes of foetal mortality. Arch Anat Cytol Pathol. 1996;44:5-11.
11. Horn LC, Langner A, Stiehl P, Wittekind C, Faber R. Identification of the causes of intrauterine death during 310 consecutive autopsies. Eur J Obstet Gynecol Reprod Biol. 2004;113:134-138.
12. Incerpi MH, Miller DA, Samadi R, Settlage RH, Goodwin TM. Stillbirth evaluation: what tests are needed? Am J Obstet Gynecol. 1998;178:1121-1125.
13. Rayburn W, Sander C, Barr M, Jr., Rygiel R. The stillborn fetus: placental histologic examination in determining a cause. Obstet Gynecol. 1985;65:637-641.
14. RCOG. Fetal and perinatal pathology. Report of a working party. 2001. London.
15. Bove KE. Practice guidelines for autopsy pathology: the perinatal and pediatric autopsy. Autopsy Committee of the College of American Pathologists. Arch Pathol Lab Med. 1997;121:368-376.
16. Langston C, Kaplan C, Macpherson T et al. Practice guideline for examination of the placenta: developed by the Placental Pathology Practice Guideline Development Task Force of the College of American Pathologists. Arch Pathol Lab Med. 1997;121:449-476.
17. Kloosterman GJ. On intrauterine growth. The significance of prenatal care. Int J Gynaecol Obstet. 1970;895-912.
18. Pinar H, Sung CJ, Oyer CE, Singer DB. Reference values for singleton and twin placental weights. Pediatr Pathol Lab Med. 1996;16:901-907.
19. Fox H. Pathology of the Placenta. Second edition. London: Saunders Company; 1997.
20. CESDI. The confidential enquiry into stillbirths and deaths in infancy. 1-163. 1993.

21. Baird D, Walker J, Thomson AM. The causes and prevention of stillbirths and first week deaths. III. A classification of deaths by clinical cause; the effect of age, parity and length of gestation on death rates by cause. J Obstet Gynaecol Br Emp. 1954;61:433-448.

22. Hey EN, Lloyd DJ, Wigglesworth JS. Classifying perinatal death: fetal and neonatal factors. Br J Obstet Gynaecol. 1986;93:1213-1223.

23. Hovatta O, Lipasti A, Rapola J, Karjalainen O. Causes of stillbirth: a clinicopathological study of 243 patients. Br J Obstet Gynaecol. 1983;90:691-696.

24. Galan-Roosen AE, Kuijpers JC, van der Straaten PJ, Merkus JM. Fundamental classification of perinatal death. Validation of a new classification system of perinatal death. Eur J Obstet Gynecol Reprod Biol. 2002;103:30-36.

25. Morrison I, Olsen J. Weight-specific stillbirths and associated causes of death: an analysis of 765 stillbirths. Am J Obstet Gynecol. 1985;152:975-980.

26. Abudu O, Akinkugbe A. Clinical causes and classification of perinatal mortality in Lagos. Int J Gynaecol Obstet. 1982;20:443-447.

27. Bhakoo ON. Wigglesworth's simplified classification of perinatal deaths. Indian J Pediatr. 1986;53:669.

28. el Zibdeh MY, Al Suleiman SA, Al Sibai MH. Perinatal mortality at King Fahd Hospital of the University Al-Khobar, Saudi Arabia. Int J Gynaecol Obstet. 1988;26:399-407.

29. Elamin S, Langhoff-Roos J, Boedker B, Ibrahim SA, Ashmeig AL, Lindmark G. Classification of perinatal death in a developing country. Int J Gynaecol Obstet. 2003;80:327-333.

30. Erdem G. Perinatal mortality in Turkey. Paediatr Perinat Epidemiol. 2003;17:17-21.

31. Settatree RS, Watkinson M. Classifying perinatal death: experience from a regional survey. Br J Obstet Gynaecol. 1993;100:110-121.

32. Bound JP, Butler NR, Spector WG. Classification and causes of perinatal mortality. Br Med J. 1956;12:1191-1196.

33. Bound JP, Butler NR, Spector WG. Classification and causes of perinatal mortality. II. Br Med J. 1956;44:1260-1265.

34. Butler NR, Alberman ED. Perinatal problems: the second report of the 1958 British Perinatal Mortality Survey. 1969. Edinburgh, E & S Livingstone Ltd.

35. Butler NR, Bonham DG. Perinatal mortality: the first report of the 1958 British Perinatal Mortality Survey. 1963. Edinburgh, E & S Livingstone Ltd.

36. Alonso A, Soto I, Urgelles MF, Corte JR, Rodriguez MJ, Pinto CR. Acquired and inherited thrombophilia in women with unexplained fetal losses. Am J Obstet Gynecol. 2002;187:1337-1342.

37. Khong TY, Chambers HM. Alternative method of sampling placentas for the assessment of uteroplacental vasculature. J Clin Pathol. 1992;45:925-927.

38. Heinonen S, Taipale P, Saarikoski S. Weights of placentae from small-for-gestational age infants revisited. Placenta. 2001;22:399-404.

39. Erwich JJ. Classification of stillbirth: cause, condition, or mechanism? BMJ. 2005;331:1269-1270.

40. Eskes, M, Van Diem, MTh. National Perinatal Audit Study. 231. 2005. Diemen, College voor Zorgverzekeringen.

Chapter 3

A multilayered approach for the analysis of perinatal mortality using different classification systems

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ABSTRACT

Many classification systems for perinatal mortality are available, all with their own strengths and weaknesses: none of them has been universally accepted. We present a systematic multilayered approach for the analysis of perinatal mortality based on information related to the moment of death, the conditions associated with death and the underlying cause of death, using a combination of representatives of existing classification systems. We compared the existing classification systems regarding their definition of the perinatal period, level of complexity, inclusion of maternal, fetal and/or placental factors and whether they focus at a clinical or pathological viewpoint. Furthermore, we allocated the classification systems to one of three categories: 'when', 'what' or 'why', dependent on whether the allocation of the individual cases of perinatal mortality is based on the moment of death ('when'), the clinical conditions associated with death ('what'), or the underlying cause of death ('why'). A multilayered approach for the analysis and classification of perinatal mortality is possible by using combinations of existing systems; for example the Wigglesworth or Nordic Baltic ('when'), ReCoDe ('what') and Tulip ('why') classification systems. This approach is useful not only for in depth analysis of perinatal mortality in the developed world but also for analysis of perinatal mortality in the developing countries, where resources to investigate death are often limited.

INTRODUCTION

Classification of perinatal mortality can reveal trends in numbers as well as causes of mortality, it can help in audit of perinatal health management by analysis of substandard factors in the care process and it can direct attention towards issues for prevention and research. Different classification systems have been designed for different reasons with different approaches, definitions, levels of complexity and availability of guidelines. Here we present a systematic multilayered approach for the analysis of perinatal mortality based on information related to the moment of death, the conditions associated with death and the underlying cause of death.

Analysis of perinatal mortality and the use of classification systems

Perinatal mortality can be evaluated by analysis of individual mortality cases or as groups of mortality in a certain hospital, region or country.

When considering individual cases of perinatal mortality the main goal is to reveal the cause of death. To assign a cause of death insight in the pathophysiology is needed. This pathophysiology is complex and involves maternal, fetal, and/or neonatal as well as placental factors. In order to assign a cause of death these factors should be addressed together. Analysis usually includes an extensive evaluation of the clinical conditions and the chain of events leading to death, including diagnostic investigations such as blood tests, autopsy and placental examination. As a result the bereaved parents can be specifically counselled about their loss and possible preventive options for future pregnancies.

Additional analysis comprises cohort analysis of the cases. The cases can be categorised in classification systems in order to reveal trends in mortality and to serve prevention and audit of perinatal care. Requirements for analysis of trends of perinatal mortality are: a universally used classification system for all participating care providers and inclusion of all perinatal mortality cases that meet the definition of the perinatal period in the analysis. This in turn requires a complete perinatal mortality registration. The inadequacies in the perinatal mortality registration have been described elsewhere.[1] Apart from the registration problem there is also the lasting discussion on perinatal period definitions; there are marked differences in these definitions in and between countries hampering an adequate comparison of perinatal mortality.[2;3]

Two perinatal mortality classification systems and their modifications are widely used throughout the world: the 'Aberdeen' and the 'Wigglesworth' classifications.[4-6] Although the two systems have been amended, the modifications and originals allow partial or complete comparison considering the consistency in categories between the systems.[7;8] Including the 'Aberdeen' and 'Wigglesworth'

Table 1. Background, strategy and perinatal inclusions

Author, year	Version	When, what, why	Aim
Baird[23], 1954	* 'Aberdeen'	what	1
Bound [24;25], 1956	*	what	1
BPMS: Butler[26], 1963	Bound	what	1
Fairweather[27], 1966	*	what	3
BPMS: Baird[28], 1969	Baird	what	1
Low[29], 1970	*	what	3
Low[30], 1971	*	what	4
Knutzen[31], 1975	*	what	1
Naeye[32], 1977	Cause of death	what	1
McIlwaine[33], 1979	Baird	what	2,4
Chang[34], 1979	*	what	1,3
Wigglesworth[13], 1980	*'Wigglesworth'*	when	1
Autio-Harmainen[35], 1983	Cause of death	what	4
Hovatta[36], 1983	Cause of death	what	4
Morrison[37], 1985	Baird	what	1,2
Cole[38], 1986	Baird	what	1
Hey[8], 1986	Wigglesworth	what	3
Hey[8], 1986	Bound	what	3
Whitfield[7], 1986	Baird	what	1,3
Pattinson[39], 1989	Baird	what	1
Lammer[40], 1989 [A]	*	what	4
Keeling[41], 1989	Wigglesworth	what	3,4
Cole[42], 1989 [A]	Wigglesworth 'ICE'	what	3
Alberman[43], 1994 [A]	Wigglesworth	what	1,3
CESDI[44], 1993	Wigglesworth	what	1
Langhoff Roos[14], 1996	*'Nordic Baltic'*	when	1,2
Alberman[45], 1997 [A]	Wigglesworth	what	1,3
Winbo[46], 1998 [A]	Wigglesworth 'NICE'	what	3
Yeo[47], 1998	Baird *'KKH Stillbirth'*	what	1
Allessandri[48], 2001	*	what	4
Galan[49], 2002	*	why	1
Chan[50], 2004	Baird *'PSANZ-PDC'*	what	1,3,4
Chan[50], 2004	Baird *'PSANZ-NDC'*	what	1,3
Gardosi[15], 2005	*'ReCoDe'*	what	1
Korteweg[11], 2006	*'Tulip'*	why	1

Version: name of the system, *: original classification system. The original author has been mentioned in case a system has been modified: Baird, Wigglesworth or Bound. Cause of death: originally published as an overview of causes of death in a certain area or hospital. When, what, why: analysis of the moment of death (when?), the clinical conditions associated with death (what?), and the underlying cause of death (why?). Aim: 1. serve prevention; 2. study perinatal mortality in a hospital or certain area,; 3. develop a classification itself; 4. other (for example: financial reasons). Strategy: main type of diagnostics applied in order to allocate the cases to a category in the system.

Strategy	Entity	Definition SB	Definition NND	Categories
2	1	ns	<7 days	main: 8 sub: 0
1	2	>28 wk	<7 days	main: 11 sub: 0
1	2	>28 wk	<7 days	main: 17 sub: 0
3	3	ns	ns	main: 16 sub: 12
3	1	>28 wk	<7 days	main: 10 sub: 18
3	2	>20 wk	ns	main: 8 sub: 0
3	2	>20 wk	<28 days	main: 8 sub: 16
2	3	>500 g	<7 days	main: 9 sub: 5
1	2,4	>20 wk	<28 days	main: 20 sub: 0
2	3	ns	ns	main: 9 sub: 28
2	2	>20 wk/> 400g	<28 days	main: 2 sub: 26
1	2	ns	ns	main: 5 sub: 0
1	2	-	<28 days	main: 6 sub: 0
3	3,4	>26 wk	-	main: 10 sub: 10
3	1,4	>20 wk	-	main: 7 sub: 9
2	1	>22 wk/>500 g	<28 days	main: 10 sub: 19
1	2	ns	ns	main: 6 sub: 0
1	2	ns	ns	main: 11 sub: 18
2	3	>20 wk	<1 year	main: 12 sub: 24
2	3	>500g	<1 year[B]	main: 12 sub: 8
3	3	>20 wk/350g	-	main: 6 sub: 0
1	2	ns	ns	main: 5 sub: 0
3	2	-	<1 year	main: 8 sub: 0
3	3	-	<28 days	main: 4 sub: 10
1	2	>20wk	<1year	main: 10 sub: 0
4	2	>28 wk/1 kg	<7 days	main: 13 sub: 0
1	2	>24 wk	-	main: 3 sub: 10
2	2	>28 wk	<28 days	main: 13 sub: 0
2	2	>28wk	-	main: 14 sub: 0
3	2	>400 g	ns	main: 10 sub: 18
3	3,4	>500 g	<7 days	main: 7 sub: 17
2	1	ns	ns	main: 11 sub: 19
3	2	-	ns	main: 7 sub: 13
3	3,4	ns	-	main: 9 sub: 39
3	3,4	>16wk	< 6months	Main: 6 sub: 42

1. pathology results 2. obstetrical or clinical results 3. combined clinico-pathology results; 4. epidemiological. Entity: classifications by individual factors. 1. mostly maternal; 2. mostly fetal; 3. maternal and fetal; 4. including adequate placental subcategories. Definitions: Definitions of inclusion period in the system: ns: not stated; wk. weeks; SB: stillbirth; NND: neonatal death. Categories: the numbers of main- and subcategories as published in the articles.
[A]Computer system [B]Population follow up in this study was until hospital discharge (<1 year), further follow up in the South African situation was difficult.

classifications, 35 systems have been introduced (published in English since 1954, introduced as a new or modified classification system, or referred to it as such by others, not mainly focusing at suboptimal care). Of these systems 20 focus on either pathological information or clinical details (Table 1). Half of the systems aim at classifying the underlying cause of death. However, the underlying cause of death, mechanism of death, clinical conditions and risk factors are often intermingled.[9] Some systems are brief and easy to use with only few categories where others are more detailed and more complex to use (Table 1). Clear uniform definitions and guidelines for classification are incomplete or not described in more than half of the systems. Seven of the analysed systems have been developed for stillbirths only, four systems for neonatal deaths only and 25 systems perinatal mortality as a group (Table 1).

As stated, the pathophysiology of perinatal death is complex and factors involving the mother, fetus/neonate and placenta should be addressed together. Only six systems address all these factors (Table 1). No single system is generally accepted for its use and each system has its own strengths and weaknesses.[10;11]

When, what, why

Recently Smith et al. have stated that the analysis of perinatal mortality requires a systematic approach.[12] This systematic approach should in our opinion include: analysis of the moment of death, of the clinical conditions associated with death and analysis of the underlying cause of death. The possibilities to complete this proposed approach is dependent on the resources available for the postmortem investigations.

The moment of death (antepartum, intrapartum and neonatal) and also the gestational age at death are important factors that reveal <u>when</u> death occurred. The Wigglesworth[13] and Nordic Baltic[14] (Table 2) classification systems for example focus at the moment of mortality (except for the category of lethal congenital malformations in both systems). These systems are easy to use as the postmortem analysis only requires clinical details considering the moment of death and macroscopic fetal examination to allocate cases to the categories.

If classification is supposed to serve in counselling, prevention or audits it is essential to classify associated clinical conditions and underlying cause of death as well. The ReCoDe classification[15] for example seeks to establish the most relevant conditions at death taking into account mother, fetus and placenta, which explains <u>what</u> happened (Table 3). The postmortem analysis for case allocation to such a system requires more details: analysis of the medical and obstetric history, the clinical course and macroscopic examination of the fetus and placenta. Autopsy and histopathological examination of the placenta are desirable, although not always necessary to explain what happened.

Table 2. 'When'

Wigglesworth[13]	
Code	**Classification**
1	Normally formed macerated stillbirth
2	Congenital malformations
3	Conditions associated with immaturity
4	Asphyxial conditions developing in labour
5	Specific conditions other than above
Nordic Baltic[14]	
Code	**Classification**
I	Fetal malformation
II	Antenatal death, single growth retarded fetus ≥ 28 weeks of gestation
III	Antenatal death, single fetus ≥ 28 weeks of gestation
IV	Antenatal death, before 28 weeks of gestation
V	Antenatal death, multiple pregnancy
VI	Intrapartum death after admission (≥ 28 weeks of gestation)
VII	Intrapartum death after admission (before 28 weeks of gestation)
VIII	Neonatal death 28-33 weeks of gestation and Apgar score >6 after 5 min
IX	Neonatal death 28-33 weeks of gestation and Apgar score <7 after 5 min
X	Neonatal death ≥ 34weeks of gestation and Apgar score >6 after 5 min
XI	Neonatal death ≥ 34weeks of gestation and Apgar score <7 after 5 min
XII	Neonatal death before 28 weeks of gestation
XIII	Unclassified

Clinical conditions however, do not necessarily explain why perinatal death occurred. The reason for death is the underlying cause of death, defined as the initial demonstrable pathophysiological entity initiating the chain of events that has irreversibly led to death. The Tulip classification[11] for example classifies these underlying causes and also the mechanism of death and contributing factors (Table 4). The postmortem analysis for these systems requires as much information as possible for establishing the underlying causes, based on clinical findings and diagnostic test results, preferably including autopsy and histopathological placental examination.

We agree with Gardosi et al that clinical conditions give insight into the scenario resulting in death. However, if (classification of) the underlying cause of death is added more insight is given. For example, in the Tulip classification 27.6% of cases (n=134) in the placenta cause of death group were small for gestational age at birth, versus 8.7% (n=42) in the other cause of death groups, illustrating diversity in distribution of small for gestational age fetuses between the cause of death groups.[16]

We allocated the published perinatal mortality classification systems to one of three categories, dependent on whether the allocation of the individual cases of perinatal mortality is based on the moment of death ('when'), the clinical conditions associated with death ('what'), or the underlying cause of death ('why') (Table 1).

Table 3. 'What'

ReCoDe[15]		
Classification	**Code**	**Sub classification**
A : Fetus	1	Lethal congenital anomaly
	2	Infection 2.1 chronic 2.2 Acute
	3	Non-immune hydrops
	4	Isoimmunisation
	5	Fetomaternal haemorrhage
	6	Twin-twin transfusion
	7	Fetal growth restriction (customised weight centiles)
B: Umbilical cord	1	Prolapse
	2	Constricting loop or knot[a]
	3	Velamentous insertion
	4	Other
C: Placenta	1	Abruptio
	2	Praevia
	3	Vasa praevia
	4	Other "placental insufficiency"[b]
	5	Other
D: Amniotic Fluid	1	Chorioamnionitis
	2	Oligohydramnios[a]
	3	Polyhydramnios[a]
	4	Other
E: Uterus	1	Rupture
	2	Uterine anomalies
	3	Other
F: Mother	1	Diabetes
	2	Thyroid diseases
	3	Essential hypertension
	4	Hypertensive diseases in pregnancy
	5	Lupus or antiphospholipid syndrome
	6	Cholestasis
	7	Drug misuse
	8	Other
G: Intrapartum	1	Asphyxia
	2	Birth trauma
H: Trauma	1	External
	2	Iatrogenic
I: Unclassified		No relevant condition identified
		No information available

PSANZ NDC[50]	
Code	**Classification**
1	Congenital abnormality
2	Extreme prematurity
3	Cardio-respiratory disorders
4	Infection
5	Neurological
6	Gastrointestinal (Necrotising enterocolitis)
7	Other (SIDS, accidents)

[a]If severe enough to be considered relevant [b]Histological diagnosis

Table 4. 'Why'

TULIP[11]			
Code	**Classification**	**Subclassification**	**Specification**
1.1.1	Congenital anomaly	Chromosomal defect	Numerical
1.1.2			Structural
1.1.3			Microdeletion/uniparental disomy
1.2.1		Syndrome	Monogenic
1.2.2			Other
1.3		Central nervous system	
1.4		Heart and circulatory system system	
1.5		Respiratory system	
1.6		Digestive system	
1.7		Urogenital system	
1.8		Musculoskeletal system	
1.9		Endocrine/metabolic system	
1.10		Neoplasm	
1.11.1		Other	Single organ
1.11.2			Multiple organ
2.1	Placenta	Placental bed pathology	
2.2.1		Placental pathology	Development
2.2.2			Parenchyma
2.2.3			Localisation
2.3		Umbilical cord complication	
2.4		Not otherwise specified	
3.1	Prematurity	PPROM	
3.2		Preterm labour	
3.3		Cervical dysfunction	
3.4		Iatrogenous	
3.5		Not otherwise specified	
4.1	Infection	Transplacental	
4.2		Ascending	
4.3		Neonatal	
4.4		Not otherwise specified	
5.1	Other	Fetal hydrops of unknown origin	
5.2.1		Maternal disease	Infection
5.2.2			Blood type incompatibility
5.2.3			Diabetes mellitus
5.2.4			Hyperthyroidism
5.2.5			Other
5.3.1		Trauma	Maternal
5.3.2			Fetal
5.4		Out of the ordinary	
6.1	Unknown	Despite thorough investigation	
6.2		Important information missing	

As approximately 60% of stillbirth cases can be explained by placental causes it is not possible to classify the underlying cause of death in systems that do not have adequate subcategories for placental causes.[16;17] When terms as hypoxia, small for gestational age or (antepartum) haemorrhage were used, we considered the system to allocate a case primarily on the clinical condition associated with death ('what') and not on the underlying cause (Table 1).

Multilayered approach

We propose a systematic multilayered approach for the analysis of perinatal mortality based on answers related to the moment of death, the conditions associated with death and the underlying cause of death. In our proposal we use existing classification systems considering the fact that all systems have strengths and weaknesses and that between persons, hospitals and regions different preferences for classification systems apply. We do not think that a single perfect system will be developed, but a well considered combination of representatives of existing systems can approach perfection. For purpose of international comparison a (standard) combination of existing systems would be preferable.

In our opinion, systems preferably combine stillbirths and neonatal deaths for the proposed multilayered approach as the same underlying causes apply.[11] The main difference between stillbirths and neonatal deaths is the different organ system that gives expression to the underlying cause of death. For example: the organ system responsible for oxygen supply in the fetus is the placenta, in neonates the lungs are responsible. The underlying cause is independent of these differences as it is the initial step in the chain of events resulting in death. Abruption of the placenta for example can cause intrauterine death and it can also cause neonatal death. The mechanisms and clinical conditions between stillbirth and neonatal death however differ; in stillbirth cases the mechanism is placental insufficiency, the clinical condition is antepartum haemorrhage, in case of neonatal death the mechanisms is respiratory insufficiency, the clinical condition is respiratory distress syndrome.

Dependent on the working area and availability of resources (developing versus developed countries and secondary versus tertiary hospital), complete analysis is often not possible. For example in the developing countries with limited resources for investigation of death, the best possible analysis may be the analysis of the moment of death and the subsequent use of one of the applicable classification systems only.[18] The preventive strategies can then be focused at timing of care, for example improved intrapartum fetal monitoring or better facilities for neonatal resuscitation. However in the developed world this analysis is insufficient and one would like to analyse the other layers of our proposed approach as well. With such

a complete analysis of perinatal mortality many details will be available for the development of preventive strategies, audit and research.
Ideally, a computerised multilayered system can be developed in order to combine in which period death occurred, what went wrong and under what circumstances using an algorithm that analyses every case in a standardised manner and gives insight into non-obvious associations.

Unknown and unexplained causes of perinatal mortality

Current use of classifications consistently report of about two thirds of perinatal mortality as being unexplained or unknown.[11;15;19-21] A large group of unexplained or unknown cases is often due to design of the system itself and lack of amendment of the system to present insight into pathophysiology of perinatal mortality. Due to differences in definition, it is difficult to compare the percentages of unexplained or unknown cases in the different systems. Moreover the problem is that these categories have little consequence and, when aiming at preventive strategies for conditions or causes they will not result in change of management.
Systems that classify the underlying cause of death require an extensive analysis of cases for optimal use, especially the autopsy and histopathologic placental examinations, as mentioned earlier in this manuscript. Perinatal autopsy rates in many developed countries however have shown a diminishing trend mainly because lack of consent, although placental examination is usually allowed.[22] In developing countries the perinatal autopsy rates are also low because the facilities for the autopsy are only available in larger hospitals and in general the autopsy does not have medical priority. Placental examination is usually not performed either, among other reasons for the risk of spread of contagious, potential lethal, infections to the examiners, such as HIV. With incomplete analysis, the underlying cause may remain unknown. For systems that classify the underlying cause of death it can be useful to define 'unknown despite thorough investigations' and 'unknown with missing important information' in order to give insight in the numbers of unknown causes due to the low autopsy rates.
The concept of our multilayered approach is particularly helpful when the underlying cause of death (with or without thorough investigations) remains unknown. The analyis of the clinical scenario with the maternal, fetal and placental conditions in that period does provide clues for preventive possibilities in the future. We used the ReCoDe (Table 3) as an example for systems that classify the clinical conditions, these systems reduce the predominance of cases formerly categorised as unknown when only classified in a system for the underlying cause of death. When in addition to these conditions the moment of death of the unknown causes is included, information can be provided considering time related conditions and the subsequent possibilities for interventions.

Table 5. Appendix. Case examples

Example 1		
28 years old, G1P0, delivery at 27 weeks of gestation, girl, 560 grams, died in utero.		
When?	Antepartum, before 28 weeks.	Nordic Baltic category VI: Antenatal death, before 28 weeks of gestation. Wigglesworth category 1: normal formed macerated stillbirth.
What?	Intra uterine growth restriction, preeclampsia and placental infarctions.	ReCoDe category A7: Fetus, fetal growth restriction, category C4: other placental insufficiency and category F4: Hypertensive disease in pregnancy.
Why?	Placenta bed pathology (infarction).	TULIP category 2.1: Placental bed pathology.
In this example all systems classify details of this case that are useful for evaluation and prevention. No system allocates a (common) case like this in a category as "other" or "unknown".		
Example 2		
36 years old, G2P1, delivery at 37 weeks of gestation, boy 2950 grams died in utero.		
When?	Antepartum, 37-40 weeks of gestation.	Nordic Baltic category III: Antenatal death, single fetus > 28 weeks of gestation. Wigglesworth category 1: normal formed macerated stillbirth.
What?	Maternal diabetes, velamentous insertion .	ReCoDe category B3: Umbilical cord, velamentous insertion and category F1: Maternal, diabetes.
Why?	No cause could be determined after autopsy and histopathological placental examination.	TULIP category 6.1: Unknown, despite thorough investigation.
In this example the cause of death remains unknown but the circumstances provide clues for prevention. The perinatal period can be evaluated including the (combination of) conditions.		
Example 3		
32 years old, G1P0, delivery at 40 weeks, boy 3220 g died three hours after birth.		
When?	In the neonatal period within 24 hours.	Nordic Baltic category XI: Neonatal death > 34 weeks of gestation and Apgar score < 7 after 5 min. Wigglesworth category 4: asphyxial conditions developing in labour.
What?	Placental abruption, mild pregnancy induced hypertension.	ReCoDe/PSANZ NDC: category C1 Placenta abruption, category F4 hypertensive disease in pregnancy and category 7: Other.
Why?	Placental bed pathology (abruption)	TULIP category 2.1: Placental bed pathology.
In this example a neonate dies of a placental cause. The ReCoDe has originally been developed for stillbirths only, but in our opinion it provides a complete view on perinatal deaths when combined with neonatal PSANZ.		

Approach for perinatal audit studies

At present for a regional audit study of perinatal mortality we use such a systematic multilayered approach. For when death occurred we register the gestational age at delivery and whether death occurred antepartum (subcategories for gestational ages are used), intrapartum or in the neonatal period (subcategories are used for death within 24 hours, death from 24 hours until one week and death from one week until four weeks of life). To classify what happened the clinical conditions

associated with perinatal mortality an amended non-hierarchical ReCoDe system is used in which we register as many items as applicable. For the clinical conditions in neonatal cases we added the PSANZ neonatal death categories as the ReCoDe has been developed for stillbirths only (Table 3). For the classification of the underlying cause of death the Tulip classification is used (Table 4). To demonstrate the benefit of such a multilayered approach for audit three case examples are provided (Table 5). The examples illustrate that with the subsequent use of representative classification systems maximum information is retained per case. Annual reports of mortality per hospital or region can summarise the figures of the selected classification systems to observe yearly trends. Subsequently additional analysis with cross-tabulation of the used systems, providing details regarding the moment of death and clinical scenario in relation to the underlying causes, is then possible, if desired in relation to substandard factors in the care process that may have contributed to death.

SUMMARY AND CONCLUSIONS

In summary we present a systematic approach of the analysis of perinatal mortality using a combination of representatives of existing classification systems. From our point of view analysis of perinatal mortality should be multilayered and include answers related to the moment of death, the conditions associated with death, and the underlying cause of death. This multilayered approach is not only useful for in depth analysis of perinatal mortality in the developed world but also for analysis of perinatal mortality in the developing countries, where resources to investigate death are often limited, as it is possible to only apply one layer. Moreover, combinations of representatives of the applicable systems can provide a complete "three-dimensional" analysis that may reveal new associations between clinical conditions and causes of death in a certain perinatal period.

REFERENCES

1. Macfarlane A, Gissler M, Bolumar F, Rasmussen S. The availability of perinatal health indicators in Europe. Eur J Obstet Gynecol Reprod Biol. 2003;111 Suppl 1:S15-S32.
2. Fretts RC, Boyd ME, Usher RH, Usher HA. The changing pattern of fetal death, 1961-1988. Obstet Gynecol. 1992;79:35-39.
3. Cartlidge PH, Stewart JH. Effect of changing the stillbirth definition on evaluation of perinatal mortality rates. Lancet. 1995;346:486-488.
4. Abudu O, Akinkugbe A. Clinical causes and classification of perinatal mortality in Lagos. Int J Gynaecol Obstet. 1982;20:443-447.
5. Elamin S, Langhoff-Roos J, Boedker B, Ibrahim SA, Ashmeig AL, Lindmark G. Classification of perinatal death in a developing country. Int J Gynaecol Obstet. 2003; 80:327-333.
6. Settatree RS, Watkinson M. Classifying perinatal death: experience from a regional survey. Br J Obstet Gynaecol. 1993;100:110-121.
7. Whitfield CR, Smith NC, Cockburn F, Gibson AA. Perinatally related wastage--a proposed classification of primary obstetric factors. Br J Obstet Gynaecol. 1986; 93:694-703.
8. Hey EN, Lloyd DJ, Wigglesworth JS. Classifying perinatal death: fetal and neonatal factors. Br J Obstet Gynaecol. 1986;93:1213-1223.
9. Erwich JJ et al. Classification of stillbirth: cause, condition, or mechanism? BMJ. 2005; 331:1269-1270.
10. Silver RM, Varner MW, Reddy U, Goldenberg R, Pinar H, Conway D et al. Work-up of stillbirth: a review of the evidence. Am J Obstet Gynecol. 2007;196:433-444.
11. Korteweg FJ, Gordijn SJ, Timmer A, Erwich JJ, Bergman KA, Bouman K et al. The Tulip classification of perinatal mortality: introduction and multidisciplinary inter-rater agreement. Br J Obstet Gynaecol. 2006;113:393-401.
12. Smith GC, Fretts RC. Stillbirth. Lancet. 2007;370:1715-1725.
13. Wigglesworth JS. Monitoring perinatal mortality. A pathophysiological approach. Lancet. 1980;2:684-686.
14. Langhoff-Roos J, Borch-Christensen H, Larsen S, Lindberg B, Wennergren M. Potentially avoidable perinatal deaths in Denmark and Sweden 1991. Acta Obstet Gynecol Scand. 1996;75:820-825.
15. Gardosi J, Kady SM, McGeown P, Francis A, Tonks A. Classification of stillbirth by relevant condition at death (ReCoDe): population based cohort study. BMJ. 2005;331:1113-1117.
16. Korteweg FJ, Gordijn SJ, Timmer A, Holm JP, Ravise JM, Erwich JJ. A Placental Cause of Intra-uterine Fetal Death Depends on the Perinatal Mortality Classification System Used. Placenta. 2008;29:71-80
17. Horn LC, Langner A, Stiehl P, Wittekind C, Faber R. Identification of the causes of intrauterine death during 310 consecutive autopsies. Eur J Obstet Gynecol Reprod Biol. 2004;113:134-138.
18. Raghuveer G. Perinatal deaths: relevance of Wigglesworth's classification. Paediatr Perinat Epidemiol. 1992;6:45-50.
19. Measey MA, Charles A, d'Espaignet ET, Harrison C, Deklerk N, Douglass C. Aetiology of stillbirth: unexplored is not unexplained. Aust N Z J Public Health. 2007;31:444-449.

20. Froen JF, Arnestad M, Frey K, Vege A, Saugstad OD, Stray-Pedersen B. Risk factors for sudden intrauterine unexplained death: epidemiologic characteristics of singleton cases in Oslo, Norway, 1986-1995. Am J Obstet Gynecol. 2001;184:694-702.

21. Fretts RC. Etiology and prevention of stillbirth. Am J Obstet Gynecol. 2005;193:1923-1935.

22. Khong TY, Turnbull D, Staples A. Provider attitudes about gaining consent for perinatal autopsy. Obstet Gynecol. 2001;97:994-998.

23. Baird D, Walker J, Thomson AM. The causes and prevention of stillbirths and first week deaths. III. A classification of deaths by clinical cause; the effect of age, parity and length of gestation on death rates by cause. J Obstet Gynaecol Br Emp. 1954; 61:433-448.

24. Bound JP, Butler NR, Spector WG. Classification and causes of perinatal mortality. II. Br Med J. 1956;44:1260-1265.

25. Bound JP, Butler NR, Spector WG. Classification and causes of perinatal mortality. Br Med J. 1956;12:1191-1196.

26. Butler NR, Bonham DG. Perinatal mortality: the first report of the 1958 British Perinatal Mortality Survey. 1963. Edingburgh, E & S Livingstone Ltd.

27. Fairweather DV, Russell JK, Anderson GS, Bird T, Millar DG, Pearcy PA. Perinatal mortality in Newcastle upon Tyne 1960-62. Lancet. 1966;1(7429):140-142.

28. Butler NR, Alberman ED. Perinatal problems: the second report of the 1958 British Perinatal Mortality Survey. 1969. Edinburgh, E & S Livingstone Ltd.

29. Low JA, Boston RW, Cervenko FW. A clinical classification of the mechanisms of perinatal wastage. Can Med Assoc J. 1970;102:365-368.

30. Low JA, Boston RW, Crussi FG. Classification of perinatal mortality. Can Med Assoc J. 1971;105:1044-1046.

31. Knutzen VK, Baillie P, Malan AF. Clinical classification of perinatal deaths. S Afr Med J. 1975;49:1434-1436.

32. Naeye RL. Causes of perinatal mortality in the US Collaborative Perinatal Project. JAMA. 1977;238:228-229.

33. McIlwaine GM, Howat RC, Dunn F, Macnaughton MC. The Scottish perinatal mortality survey. Br Med J. 1979;2:1103-1106.

34. Chang A, Keeping JD, Morrison J, Esler EJ. Perinatal death: audit and classification. Aust N Z J Obstet Gynaecol. 1979;19:207-211.

35. Autio-Harmainen H, Rapola J, Hoppu K, Osterlund K. Causes of neonatal deaths in a pediatric hospital neonatal unit. An autopsy study of a ten-year period. Acta Paediatr Scand. 1983;72:333-337.

36. Hovatta O, Lipasti A, Rapola J, Karjalainen O. Causes of stillbirth: a clinicopathological study of 243 patients. Br J Obstet Gynaecol. 1983;90:691-696.

37. Morrison I, Olsen J. Weight-specific stillbirths and associated causes of death: an analysis of 765 stillbirths. Am J Obstet Gynecol. 1985;152:975-980.

38. Cole SK, Hey EN, Thomson AM. Classifying perinatal death: an obstetric approach. Br J Obstet Gynaecol. 1986;93:1204-1212.

39. Pattinson RC, De Jong G, Theron GB. Primary causes of total perinatally related wastage at Tygerberg Hospital. S Afr Med J. 1989;75:50-53.

40. Lammer EJ, Brown LE, Anderka MT, Guyer B. Classification and analysis of fetal deaths in Massachusetts. JAMA. 1989;261:1757-1762.

41. Keeling JW, MacGillivray I, Golding J, Wigglesworth J, Berry J, Dunn PM. Classification of perinatal death. Arch Dis Child. 1989;64:1345-1351.

42. Cole S, Hartford RB, Bergsjo P, McCarthy B. International collaborative effort (ICE) on birth weight, plurality, perinatal, and infant mortality. III: A method of grouping underlying causes of infant death to aid international comparisons. Acta Obstet Gynecol Scand. 1989;68:113-117.

43. Alberman E, Botting B, Blatchley N, Twidell A. A new hierarchical classification of causes of infant deaths in England and Wales. Arch Dis Child. 1994;70:403-409.

44. CESDI. The confidential enquiry into stillbirths and deaths in infancy. Report March 1992-July 1993:1-163.

45. Alberman E, Blatchley N, Botting B, Schuman J, Dunn A. Medical causes on stillbirth certificates in England and Wales: distribution and results of hierarchical classifications tested by the Office for National Statistics. Br J Obstet Gynaecol. 1997;104:1043-1049.

46. Winbo IG, Serenius FH, Dahlquist GG, Kallen BA. NICE, a new cause of death classification for stillbirths and neonatal deaths. Neonatal and Intrauterine Death Classification according to Etiology. Int J Epidemiol. 1998;27:499-504.

47. Yeo SH, Tee CS, Tan KH. Stillbirths in KK Women's and Children's Hospital 1997. Report of the KKH Stillbirth Working Group.

48. Alessandri LM, Chambers HM, Blair EM, Read AW. Perinatal and postneonatal mortality among Indigenous and non-Indigenous infants born in Western Australia, 1980-1998. Med J Aust. 2001;175:185-189.

49. Galan-Roosen AE, Kuijpers JC, van der Straaten PJ, Merkus JM. Fundamental classification of perinatal death. Validation of a new classification system of perinatal death. Eur J Obstet Gynecol Reprod Biol. 2002;103:30-36.

50. Chan A, King JF, Flenady V, Haslam RH, Tudehope DI. Classification of perinatal deaths: development of the Australian and New Zealand classifications. J Paediatr Child Health. 2004;40:340-347.

Part II

Value of diagnostic tests after intrauterine antepartum fetal death

Chapter 4

Diverse placental pathologies as the main causes of fetal death

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ABSTRACT

Objective

To estimate the occurrence of placental causes of fetal death in relation to different gestational ages and their clinical manifestations during pregnancy.

Methods

In a prospective cohort study conducted from 2002 to 2006, we studied 750 couples with singleton intrauterine fetal death after 20 weeks of gestation. Cause of death was classified according to the Dutch Tulip causes of death classification for perinatal mortality. Differences between groups for categorical data were evaluated by the Fisher exact test or $\chi 2$ test.

Results

The main causes were placental pathology (64.9%), congenital anomaly (5.3%), infection (1.9%), other (4.8%) and unknown (23.1%). The contribution of causes differed over gestational age periods. At lower gestational age, placental and unknown were the most dominant causes of death (34.8% and 41.7%, respectively) whereas at higher gestational age the relative importance of an unknown cause decreased and a placental cause increased (16.5% and 77.6%) ($P<0.001$). Placental bed pathology was observed in 33.6% of all fetal deaths, with the highest occurrence between 24 0/7 and 31 6/7 weeks and a strong decline after 32 weeks. In contrast, contribution of developmental placental pathology (17.6%) increased after 32 weeks of gestation ($P<0.001$), as did umbilical cord complications (5.2%) and combined placental pathology (5.5%). Solitary placental parenchyma pathology was less frequent (3.1%). Hypertension-related disease was observed in 16.1% (95% confidence interval (CI) 13.6-19.0) of the cohort, small for gestational age fetuses in 37.9% (95% CI 34.1- 41.7) and diabetes-related disease in 4.1% (95% CI 2.8-5.8).

Conclusion

Most fetal deaths were caused by a variety of placental pathologies. These were related to gestational age, and their clinical manifestations varied during pregnancy.

INTRODUCTION

Worldwide some 4 million stillbirths are reported officially each year. In developing countries, 1 in 200 pregnancies ends in stillbirth, defined as intrauterine fetal death or intrapartum death. The cause of death is unexplained in about two-thirds of cases.[1] There are intensifying demands on medical, political and epidemiological grounds for proper determination of the causes.[2]

The woman, the fetus and the placenta all are involved in the complex process of fetal death and, therefore, should be addressed together. The placenta can be considered the diary of pregnancy; after death it remains viable for several days. The value of examining the placenta for determining or excluding a cause of death in stillbirths is evident and varies from 28-85%.[3-5] The value depends largely on the quality of the placental reports.[6] Placental causes of death have been found in up to 60% of perinatal mortality cases[7-9] and 64% of intrauterine fetal deaths, depending on the classification system used to classify cause of death.[10] Placental causes, if included in these classification systems at all, remain nonspecific. Many of the other widely used categories of causes of death, including preeclampsia and unexplained stillbirth, are thought to be related to placental dysfunction.[10-12] To reduce the fetal death rate, we need to gain more insight into placental causes.[1,12]

Fetal death can be divided by gestational age at birth into early (20-28 weeks) or late deaths (>28 weeks).[12] No large cohort studies were available that could report on occurrence of clearly defined and classified placental causes of intrauterine fetal death during different gestational periods.

We aimed to estimate the occurrence of different placental causes related to different gestational age periods, and the clinical manifestations during pregnancy, in a large cohort of couples with fetal death. Our goal was to propose a further pathophysiologic classification of placental causes on the basis of our findings.

MATERIALS AND METHODS

In 2002, we initiated the Dutch prospective intrauterine fetal death cohort study in 50 secondary and tertiary referral hospitals in the Netherlands, serving rural as well as urban populations. Inclusion criteria were singleton intrauterine fetal deaths diagnosed antepartum (heart beat ceased before labor) after 20 weeks of gestation calculated from the last menstrual period and confirmed by ultrasonography. Pregnancy terminations and intrapartum deaths were excluded.

The study was approved by the review boards of all the hospitals, and written informed consent was obtained from all participants. The inclusion rates varied between hospitals. Reasons for exclusion were denied informed consent, patient

language barrier, logistic problems, and the doctor's reluctance to include women with intrauterine fetal death because of an already known cause of fetal death at birth. This involved deaths with placental abruption, known chromosomal abnormalities, and major congenital anomalies, which therefore probably resulted in an underrecording of such deaths in our data. Collected data included medical and obstetric history, maternal and fetal characteristics, and pregnancy and birth details. There is no international uniform diagnostic guideline for stillbirth, so we first set out a diagnostic work-up protocol after determination of intrauterine fetal death that included extensive maternal blood tests including full blood count, chemistry, viral serology, and coagulation tests performed by a central laboratory; fetal blood tests including viral serology; microbiologic cultures from woman, fetus and placenta; autopsy; placental examination; and cytogenetic analysis.

Autopsy and placental examination were performed by surgical and perinatal pathologists in the participating hospitals. We urged pathologists to follow the study protocol, which was based on the guidelines published by The Royal College of Obstetricians and Gynaecologists and the Royal College of Pathologists,[13] and the College of American Pathologists.[14,15] All of the examined placentas also were studied for histopathology.

Five gestational age periods were defined in: 20 0/7-23 6/7, 24 0/7-27 6/7, 28 0/7-31 6/7, 32 0/7-36 6/7 and 37 0/7-43. When the study was initiated in 2002, customized growth charts were not available. Fetal growth percentiles for birth weight by gestational age at time of diagnosis of intrauterine fetal death therefore were calculated according to Kloosterman's growth charts, commencing at 25 weeks of gestation.[16] Small for gestational age (SGA) was defined as birth weight less than the 10th percentile. Definitions for hypertension-related disease (chronic hypertension, pregnancy-induced hypertension, preeclampsia, hemolysis, elevated liver enzymes, low platelets syndrome, and superimposed conditions) were based on recommendations by the International Society for the Study of Hypertension in Pregnancy.[17]

Adjudication of cause of death

The cause, mechanism, origin, and contributing factors of death were classified independently according to the Tulip classification[8] (with the use of patient information sets) by experienced, multidisciplinary panel members consisting of two obstetricians (JJE, JPH), an obstetric resident (FJK), and a paediatric pathologist (AT). Afterwards, central panel meetings resulted in consensus after discussion. The cause was defined as the initial, demonstrable pathophysiologic entity initiating the chain of events that had led irreversibly to death. The mechanism was defined as organ failure that was not compatible with life, initiated by the cause that had led directly to death. Origin of mechanism was defined

as the explanation of the mechanism of death. Risk factors such as smoking or hypertension, defined as other known contributing factors to death, were also identified. Only one underlying cause of death could be allocated per case.

Subgroups of placental cause of death were defined as described previously.[8,10] These were placental bed pathology and developmental pathology. Placental bed pathology involves inadequate spiral artery remodeling or spiral artery pathology or both leading to maternal vascular underperfusion. Pathology consistent with placental bed pathology included placental abruption (a clinical diagnosis supported by placental examination) and significant infarction (in preterm cases, any placental infarction; in term cases, extensive infarction [>10% of the placental area]). Placental bed pathology as a cause was allocated if the percentage of infarctions in the parenchyma in relation to the weight of the placenta was regarded likely to cause death. In a term placenta of appropriate weight, at least 30% infarctions was regarded plausible to cause death. This is in accordance with others.[18]

Developmental pathology is a morphologic abnormality due to abnormal development. Placental hypoplasia was defined as an absolute too low placental weight less than the 10th percentile or a too low placenta:birth weight ratio or both.[10] Villus immaturity is a placental maturation defect with microscopy revealing deficient formation of synctiocapillary membranes[19] and was considered as a cause of death after 36 weeks gestation. Parenchyma pathology is an acquired disorder of the villi or intervillous space such as maternal floor infarction/massive perivillous fibrin deposition[20] or feto-maternal hemorrhage without obvious cause. Fetal thrombotic vasculopathy[21] was assigned as a cause of death only if there was no evidence of umbilical cord blood-flow restriction. Localization pathology is placenta praevia. Umbilical cord complication is placental pathologic confirmation of umbilical cord blood-flow restriction by macroscopy (congestion, edema, and thrombosis) or microscopy (thrombosis, intramural vascular fibrin deposition, intramural vascular calcification and hemorrhagic endovasculitis, avasculair chorionvilli) or both supported by clinical findings and excluding other causes of death. Placenta pathology not otherwise specified is a combination of placental causes when no choice could be made as to which was first in the chain of events leading to death.

Statistics

Sample size of the study was based on the ability to identify less frequent but clinically relevant causes of death (i.e 5% rather than formal hypothesis testing). To achieve sufficient precision, we considered a lower boundary of the 95% confidence interval (CI) of 3.5%. This resulted in a sample size of 750 fetal deaths. Categorical variables were expressed as counts and percentages and continuous data as a mean with standard deviation or median and ranges, with exact 95% CIs when appropriate. Differences between groups for categorical data were

evaluated by the Fisher exact test or chi-squared test. A two-tailed $P<0.05$ was considered to indicate statistical significance. Statistical analyses were performed using SAS 9 (SAS-Institute Inc., Cary, NC, USA).

RESULTS

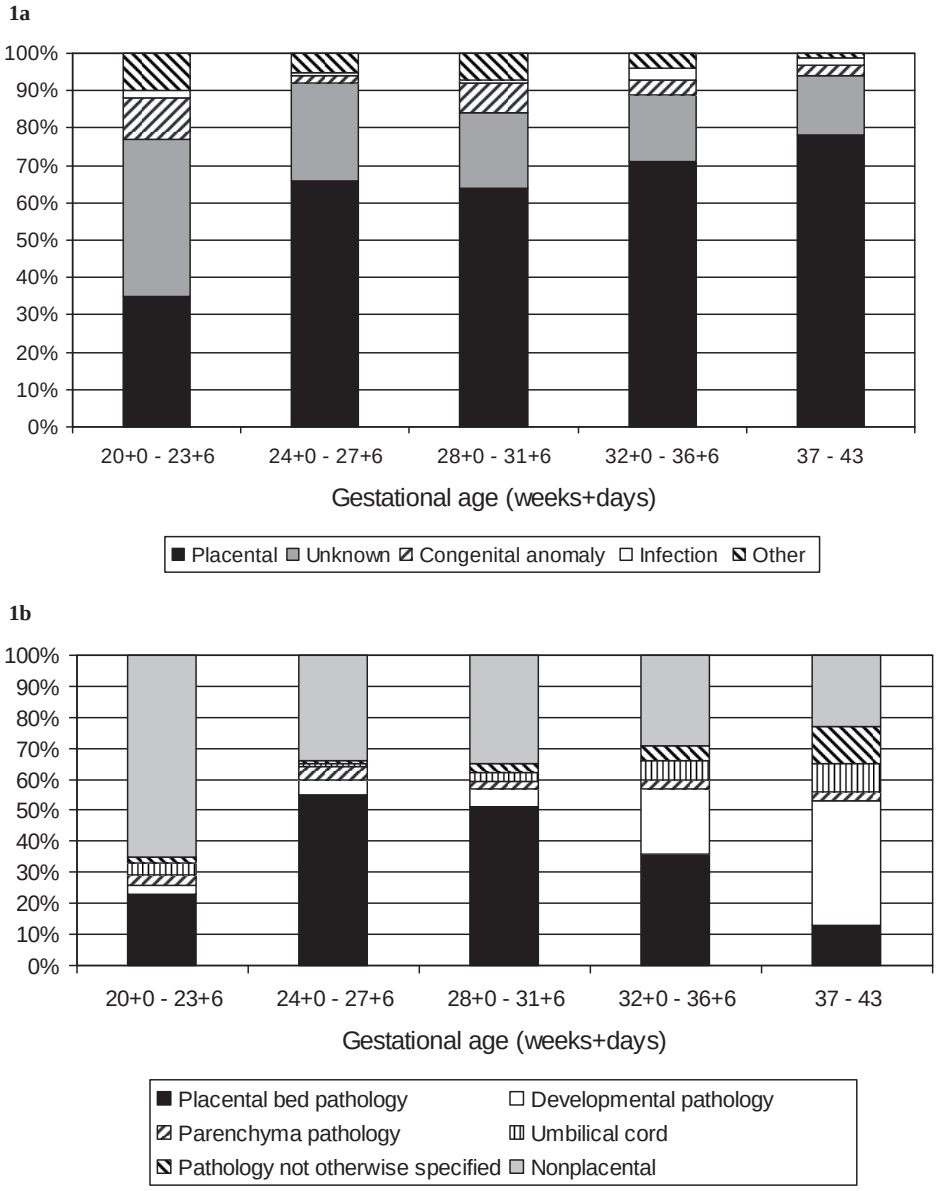
From 2002 to 2006, 750 couples and their fetal deaths were studied. The median age of the women was 31 years (range 18 to 46 years) and the median gestational age at determination of intrauterine fetal death 31 4/7 weeks (range 20 0/7 weeks to 421/7 weeks). The median fetal weight was 1470 grams (range 12 to 4630 grams). Significantly more intrauterine fetal deaths were boys (n=408) than girls (n=339; null hypothesis of equal percentages, P=0.01); sex at birth could not be determined in three cases because there was no information on cytogenetic or pathologic examination available. An autopsy was performed in 525 fetal deaths (70.0%), external macroscopic fetal examination by an expert in 24 (3.2%), and a placental examination in 736 cases (98.1%).

The main causes of death for the total cohort were placental pathology (64.9%), congenital anomaly (5.3%), infection (1.9%) and other (4.8%), in 23.1% the cause remained unknown after classification (15.9% despite thorough investigation and 7.2% because important information was missing) (Table 1). The contribution of causes of death differed over gestational age periods (Figure 1). Within each gestational age period, cause of death amounted to 100%. At lower gestational age, placental and unknown were the most dominant main causes of death (34.8% and 41.7%, respectively); at higher gestational age, the relative importance of an unknown cause decreased and placental causes increased (16.5% and 77.6%, respectively) ($P<0.0001$) (Figure 1a). Placental bed pathology, a placental subgroup, was observed in 33.6% of all deaths, with the highest occurrence between 24 and 31 6/7 weeks and with a strong decline after 32 weeks. In contrast, at the same

Table 1. Overall causes of death in a cohort of 750 intrauterine fetal deaths, according to the Tulip classification by gestational age

Cause of death n (%)	Gestational age in weeks					
	20^0-23^6	24^0-27^6	28^0-31^6	32^0-36^6	37^0 – 43	Total
Congenital anomaly	13 (11.3)	2 (1.5)	12 (8.2)	7 (4.5)	6 (2.9)	40 (5.3)
Placenta	40 (34.8)	86 (66.2)	93 (63.7)	109 (70.8)	159 (77.6)	487 (64.9)
Infection	2 (1.8)	2 (1.5)	2 (1.4)	4 (2.6)	4 (1.9)	14 (1.9)
Other	12 (10.4)	6 (4.6)	10 (6.8)	6 (3.9)	2 (1.0)	36 (4.8)
Unknown	48 (41.7)	34 (26.2)	29 (19.9)	28 (18.2)	34 (16.5)	173 (23.1)
Total	115 (100)	130 (100)	146 (100)	154 (100)	205 (100)	750 (100)

Figure 1. Distribution of overall (1a) and placental causes of death (1b) in a cohort of 750 intrauterine fetal deaths according to the Tulip classification by gestational age



time, the contribution of developmental placental pathology (17.6%) increased after 32 weeks of gestation (P<0.001, Figure 1b).

The origins of mechanism of these placental subgroups are presented in Table 2. Placental infarctions were observed in 26.7% of the total cohort (n=750) showing a peak between 24 and 32 weeks; abruption was observed in 7.3% of cases, mostly

between 28 and 37 weeks gestation. Placental hypoplasia was observed in 13.0%, mainly after 32 weeks. Villus immaturity was considered as a cause of death after 36 weeks gestation. There were, however, seven cases between 35 and 36 weeks for which this cause was also allocated owing to an explicit pathology report. Solitary placental pathology of the parenchyma was rare (3.1%) and not related to gestational age. Umbilical cord complications were observed in 5.2% of the cohort and presented mainly as strangulation in the term period. Placental pathology not otherwise specified as the cause of death (5.4%) was observed most often during the term period (P=0.02). The origins of mechanism represented various

Table 2. Origin of mechanism of placental causes according to the Tulip classification by gestational age

Origin of mechanism n (%)	**Gestational age in weeks**					
	20^0-23^6	**24^0-27^6**	**28^0-31^6**	**32^0-36^6**	**37^0 – 43**	**Total**
Placental bed pathology	**26 (22.6)**	**71 (54.6)**	**73 (50.0)**	**55 (35.7)**	**27 (13.2)**	**252 (33.6)**
Abruptio placentae	6	9	14	18	5	52 (6.9)
Infarction	20	60	58	37	22	197 (26.3)
Abruptio placentae and infarction	-	2	1	-	-	3 (0.4)
Placental pathology development	**3 (2.6)**	**7 (5.4)**	**8 (5.5)**	**32 (20.8)**	**82 (40.0)**	**132 (17.6)**
Villus immaturity	-	-	-	6	26	32 (4.3)
Hypoplasia	3	7	8	24	48	90 (12.0)
Villus immaturity and hypoplasia	-	-	-	2	6	8 (1.0)
Other; excessive bleeding	-	-	-	-	2	2 (0.3)
Placental pathology parenchyma	**4 (3.5)**	**5 (3.9)**	**3 (2.1)**	**5 (3.3)**	**6 (2.9)**	**23 (3.1)**
Fetal thrombotic vasculopathy	1	1	-	-	-	2 (0.3)
Massive perivillous fibrin deposition	2	1	1	1	2	7 (0.9)
Villitis of unknown origin	1	1	1	-	1	4 (0.6)
Intervillositis	-	1	-	-	-	1 (0.1)
Other; excessive bleeding	-	1	1	4	3	9 (1.2)
Umbilical cord complication	**5 (4.4)**	**1 (0.8)**	**4 (2.7)**	**10 (6.5)**	**19 (9.3)**	**39 (5.2)**
Knot	-	-	-	1	4	5 (0.7)
Strangulation	2	-	2	6	12	22 (2.9)
Knot and strangulation	1	-	1	2	3	7 (0.9)
Torsion	2	1	1	1	-	5 (0.7)
Placental pathology NOS*	**2 (1.7)**	**2 (1.5)**	**5 (3.4)**	**7 (4.5)**	**25 (12.2)**	**41 (5.4)**
Other nonplacental causes	**75 (65.2)**	**44 (33.8)**	**53 (36.3)**	**45 (29.2)**	**46 (22.4)**	**263 (35.1)**
Total	**115 (100)**	**130 (100)**	**146 (100)**	**154 (100)**	**205 (100)**	**750 (100)**

*NOS: not otherwise specified, combination of pathologies, data are n (%) or n

combinations of placental pathology, mainly placental hypoplasia (3.1%), villus immaturity (2.4%) and fetal thrombotic vasculopathy (2.5%) (Table 3).

The association between the clinical manifestations small for gestational age (SGA) fetuses, hypertension, and diabetes-related maternal disease observed during pregnancy for placental compared with nonplacental causes of death are shown in Table 4. Overall, we observed hypertension-related disease in 16.1% (95% CI 13.6 to 19.0) of the cohort. This was higher for women with fetal deaths caused by placental bed pathology (33.7%, 95% CI 27.9 to 39.9), compared with the groups with placental hypoplasia, other placental causes, or nonplacental causes

Table 3. Placental causes: not otherwise specified

Origin of mechanism	Gestational age in weeks and days
Abruptio placentae, hypoplasia	27+3
Abruptio placentae, villus immaturity, fetal thrombotic vasculopathy	41+5
Infarction, fetal thrombotic vasculopathy n=3	23+3, 35+3, 35+4
Infarction, placenta increta	35+0
Infarction, stricture umbilical cord	24+4
Infarction, villitis of unknown origin	40+1
Infarction, villus immaturity	36+0
Infarction, villus immaturity, amniotic web with stowage in umbilical cord	37+2
Hypoplasia, excessive bleeding	37+5
Hypoplasia, fetal thrombotic vasculopathy	38+3
Hypoplasia, fetal thrombotic vasculopathy, lymfohistiocytaire villitis	40+5
Hypoplasia, fetal thrombotic vasculopathy, massive perivillous fibrin deposition	37+1
Hypoplasia, fetal thrombotic vasculopathy, villitis of unknown origin	37+4
Hypoplasia, fetal thrombotic vasculopathy, villus immaturity n=3	37+6, 38+4, 40+1
Hypoplasia, gitter infarct	40+3
Hypoplasia, immature placenta, fetal thrombotic vasculopathy, umbilical cord strangulation	40+0
Hypoplasia, villitis of unknown origin n=5	28+6, 29+6, 31+4, 37+6, 40+5
Hypoplasia, villus immaturity, fetal thrombotic vasculopathy, villitis of unknown origin n=2	
Hypoplasia, villus immaturity, villitis of unknown origin n=2	39+6, 41+3
Hypoplasia, villus immaturity, villitis of unknown origin, umbilical knot	30+2
Hypoplasia, umbilical knot	31+2
Hypoplasia, villus immaturity, umbilical cord occlusion	41+3
Villus immaturity, excessive bleeding	41+1
Villus immaturity, fetal thrombotic vasculopathy n=5	36+3, 36+5, 40+0, 40+3, 41+0
Villus immaturity, umbilical cord occlusion	35+3
Fetal thrombotic vasculopathy, massive perivillous fibrin deposition	38+0
Intervillositis, retroplacental haematoma	20+0

Table 4. Clinical manifestations in relation to placental and nonplacental causes of death

Clinical manifestations	Cause of death			
	Placental bed pathology	Placental hypoplasia	Other placental	Non placental
Total	252 (33.6)	98 (13.1)	137 (18.3)	263 (35.1)
Hypertension-related disease	85 (33.7)	10 (10.2)	11 (8.0)	15 (5.7)
PIH	24 (9.5)	2 (2.0)	6 (4.4)	5 (1.9)
PE/ eclampsia	22 (8.7)	2 (2.0)	1 (0.7)	2 (0.8)
PIH and HELLP	3 (1.2)	-	-	-
PE and HELLP	13 (5.2)	-	-	-
Chronic hypertension	1 (0.4)	2 (2.0)	2 (1.4)	4 (1.5)
Superimposed PIH	12 (4.8)	3 (3.1)	2 (1.4)	3 (1.1)
Superimposed PE	8 (3.2)	-	-	-
Superimposed PE/HELLP	2 (0.8)	1 (1.0)	-	1 (0.4)
SGA, affected/assessed (%)	132/224 (58.9)	26/93 (28.0)	26/127 (20.5)	61/203(30.0)
Non-SGA nonhypertensive	77/224 (34.4)	67/93 (72.0)	102/127 (80.3)	190/203 (93.6)
Diabetes-related disease	5 (2.0)	9 (9.2)	7 (5.1)	10 (3.8)
Insulin-dependant diabetes	2 (0.8)	4 (4.1)	2 (1.5)	5 (1.9)
Gestational diabetes	3 (1.2)	5 (5.1)	5 (3.6)	5 (1.9)

PIH: pregnancy-induced hypertension, PE: preeclampsia, HELLP: hemolysis, elevated liver enzymes, low platelets, SGA: small for gestational age, data are n (%)

(P<0.001). Overall, 37.9% (95% CI 34.1 to 41.7) of the 647 fetal deaths after 24 weeks of gestation were SGA. This was seen more frequently in deaths caused by placental bed pathology (58.9%; 95% CI 52.2 to 65.4) than in the other subgroups (P<0.001). Still, more than one third of cases with placental bed pathology were non-SGA (41.1%) and two thirds were nonhypertensive (66.3%). Overall, 67.4% (95% confidence interval 63.6 to 71.0) of the women did not present with SGA or a hypertensive disorder, but, in the group of fetal deaths due to placental bed pathology, this proportion was lower at 34.4% (95% CI 28.2 to 41.0). Furthermore, maternal diabetes-related disease was observed in 4.1% of cases (95% CI 2.8 to 5.8), particularly in the group of fetal deaths due to placental hypoplasia. All of these placentas had a too low placenta:birth weight ratio and included five that also had an absolute too low placental weight less than the 10th percentile.

DISCUSSION

In our cohort of 750 couples and their antepartum fetal deaths, placental pathology represented 64.9% of the causes determined. The occurrence of a placental cause of death rose as gestational age increased. At preterm gestations (before 32 weeks), placental bed pathology was the main cause of death; developmental

	Total
	750 (100)
	121 (16.1)
	37 (4.9)
	27 (3.6)
	3 (0.4)
	13 (1.7)
	9 (1.2)
	20 (2.7)
	8 (1.1)
	4 (0.5)
	245/647 (37.9)
	436/647 (67.4)
	31 (4.1)
	13 (1.7)
	18 (2.4)

pathology, umbilical cord complications, and combined pathology were more common after 32 weeks gestation. The occurrence of SGA fetuses, maternal hypertension, and diabetes-related disease differed over placental subgroups.

We can partially explain differences in occurrence of placental causes of death compared with other studies by the design of the classification systems used. Not all of the systems address maternal, fetal and placental factors together, and the information required to assign a cause of death varies between systems. The cause of death, mechanism, and risk factors are often mixed up, leading to clinical conditions being allocated as a cause.[10,22] The value of our study lies in the uniform, standardized approach that we used to evaluate the fetal deaths and the strict criteria we used to allocate a placental cause of death. Placental abnormalities also may be common in live births; accordingly, it is difficult to be sure of their relevance when found in a cohort of stillbirths. Because this was not a case-control study, it was not our intention to determine differences between abnormal placental findings in intrauterine fetal deaths and live births. In our study, all cases were fetal deaths, and, in the process of adjudication of cause, not only placentas were examined; it was also other diagnostic tests and the clinical setting that led to final panel consensus regarding cause of death. However, strict panel guidelines on how to interpret placental pathology findings were used to have proper standardization. Because placentas were examined by different surgical as well as perinatal pathologists, we must presume that there is interobserver variation resulting in underestimation of placental pathologies that are relatively difficult to establish. Furthermore, it is well known that quality of at least 36% of placental reports is below standard.[6]

The largest group of placental causes of death was placental bed pathology, evident in 33.6% of all deaths. Others reported 23% of deaths in an intrauterine fetal death cohort due to placental bed pathology.[7] The origin of mechanism for the majority of fetal deaths in our placental bed pathology group was infarction (79.4%), mostly resulting in early deaths before 32 weeks gestation. Sebire et al. studied placentas in pregnancies complicated by preeclampsia and also found the percentage of infarctions to be greater at earlier gestational age.[23] Others report similar results.[24] Zhang et al. found only 10% of pregnancy-induced hypertension placentas at more than 35 weeks gestation with maternal vascular under perfusion, but the placentas were smaller.[25] Our findings and those of others suggest a different mechanism of disease in preterm pregnancies complicated by placental bed pathology than in term pregnancies. Placental abruption was seen in 7.3% of our cohort, lower than the 10-20% reported earlier.[26] The timing of most of these

deaths, expressing acute vascular insufficiency, was later than due to infarctions expressing chronic insufficiency. Earlier, abruption was found to be the most common factor contributing to fetal death after 32 weeks gestation in women with preeclampsia.[27]

Our results showed that, overall, 68% of women did not have manifest hypertension-related disease or SGA fetuses; for the placental bed pathology group, this was 34%. This implies that expression of clinical manifestations is not always predictive of the type of placental pathology causing death. SGA seems to be more a marker of placental dysfunction than causally associated with death. The extent to which fetal growth is altered by placental bed pathology probably depends on the time at onset and duration of placental damage.

The contribution of developmental placental pathology as a cause (17.6%) increased after 32 weeks of gestation, in contrast to placental bed pathology. Placental hypoplasia as the origin of mechanism represented 74.2% of this group (13.0% of all fetal deaths). Small for gestational age and hypertension-related disease both were observed less often in the developmental hypoplasia group than in the placental bed pathology group (28.0% compared with 58.9%; $p<0.001$ and 10.2% compared with 33.7%; $p<0.001$, respectively). These differences in gestational age distribution, as well as the different occurrence of SGA and hypertension-related disease in both placental subgroups, support the separate classification of these two pathologies as a cause of death and suggest a different pathogenesis.[10,22] However, some of the deaths classified in the developmental pathology-hypoplasia group might have been misclassified owing to sampling or diagnostic errors, resulting in an under-estimation of placental bed pathology due to underperfusion rather than infarction or abruption.[28,29]

Villus immaturity was found to be a cause in 7.7% of cases. This was slightly higher than the 5.7% reported by an earlier study in unselected placentas, including 2.3% associated with fetal death.[19] The risk of stillbirth associated with placental villus immaturity is 70-fold that with a normal placenta, and the risk of recurrent stillbirth is10-fold above baseline.[19] When considering fetal growth in this group, only 19% was SGA compared with 39% in the total cohort and 59% in the placental bed pathology group. Placental delivery of oxygen does not seem to be linked to that of nutrients. Acquired disorders of the placental parenchyma were not related to gestational age and were seen as solitary (3.1%) or combined with other placental causes (3.6%). Occurrence of fetal thrombotic vasculopathy was 2.8% compared with 1% reported earlier[21] and 1.2% for maternal floor infarction/massive perivillous fibrin deposition compared to 0.4% earlier.[30] Although they are infrequent, identifying these placental pathologies as the origin of mechanism is clinically relevant because some of these may be associated with a high recurrence risk and some may be amenable to preventive therapies in the future.[31]

Umbilical cord complications (5.2%) were observed mainly from mid-third trimester onward, with less fetal space in utero. This is lower than the 9-15% in stillbirths suggested by others.[7,32] Our low percentage is the result of our strict criteria: care should be used when assigning this cause because 30% of pregnancies with live birth also are complicated by nuchal cords and true knots.

In conclusion, placental pathology was found to be the main cause of fetal death in a cohort of 750 intrauterine fetal death cases. The occurrence of placental pathologies and the expression of clinical manifestations varied over gestational age periods and across placental subgroups. Based on the occurrence of our reported placental pathologies as the origin of mechanism in intrauterine fetal death, we propose amending the subgroups of placental causes of death, as defined in the Tulip classification[8] based on pathophysiology (Table 5). For unequivocal classification, we urge that all placentas be examined routinely, both macroscopically and for histopathology according to a standardized pathologic guideline. By using a more detailed classification for cause of death, health workers will be better able to counsel parents based on placental cause-specific

Table 5. The Tulip classification of perinatal mortality; proposed further subdivision of placental causes of death

1. Placental bed pathology	1. Vascular disruption (e.g. placental abruption)
	2. Vascular obstruction (e.g. infarction)
	3. Other*
	4 Combined pathology
2. Placental pathology	1. Development 1. Placental growth (e.g. hypoplasia)
	2. Maturation defect (e.g. villus immaturity)
	3. Other§ (e.g. placenta circumvallata)
	4. Combined pathology
Parenchyma	1. Maternal circulatory (e.g. MFI/MPFD†)
	2. Fetal circulatory (e.g. FTV¶)
	3. Inflammatory (e.g. villitis of unknown origin)
	4. Other‡ (e.g. fetal-maternal haemorrhage without obvious cause)
	5. Combined pathology
Localization	1. Intrauterine (e.g placenta praevia)
	2. Extrauterine
3. Umbilical cord complication	1. Extrinsic (e.g. true knot, entanglement, prolapse)
	2. Intrinsic (e.g. thrombosis, torsion)
	3. Combined pathology
4. Combined pathology (of placental main groups 1,2 or 3)	

*Other pathology consistent with maternal vascular under perfusion without infarction or abruption, §Other developmental pathology, †MFI: maternal floor infarction/MPFD: massive perivillous fibrin deposition, ¶FTV: fetal thrombotic vasculopathy, ‡Other parenchyma pathology

recurrence rates. Screening tests for impaired placentation and evaluation of interventions in these placental subgroups then might help prevent stillbirths in subsequent pregnancies.

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REFERENCES

1. Silver RM. Fetal death. Obstet Gynecol. 2007;109:153-167.
2. Buitendijk S, Zeitlin J, Cuttini M, Langhoff-Roos J, Bottu J. Indicators of fetal and infant health outcomes. Eur J Obstet Gynecol Reprod Biol. 2003;111 Suppl 1:S66-S77.
3. Burnley H, Moore I. An audit to assess the quality of necropsies performed on stillborn infants. J Clin Pathol. 2005;58:93-94.
4. Gordijn SJ, Erwich JJ, Khong TY. Value of the perinatal autopsy: critique. Pediatr Dev Pathol. 2002;5:480-488.
5. Thornton CM, O'Hara MD. A regional audit of perinatal and infant autopsies in Northern Ireland. Br J Obstet Gynaecol. 1998;105:18-23.
6. Khong TY, Gordijn SJ. Quality of placental pathology reports. Pediatr Dev Pathol. 2003;6:54-58.
7. Horn LC, Langner A, Stiehl P, Wittekind C, Faber R. Identification of the causes of intrauterine death during 310 consecutive autopsies. Eur J Obstet Gynecol Reprod Biol. 2004;113:134-138.
8. Korteweg FJ, Gordijn SJ, Timmer A et al. The Tulip classification of perinatal mortality: introduction and multidisciplinary inter-rater agreement. BJOG. 2006;113:393-401.
9. Rayburn W, Sander C, Barr M, Jr., Rygiel R. The stillborn fetus: placental histologic examination in determining a cause. Obstet Gynecol. 1985;65:637-641.
10. Korteweg FJ, Gordijn SJ, Timmer A, Holm JP, Ravise JM, Erwich JJ. A placental cause of intra-uterine fetal death depends on the perinatal mortality classification system used. Placenta. 2008;29:71-80.
11. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005;308:1592-1594.
12. Smith GC, Fretts RC. Stillbirth. Lancet. 2007;370:1715-1725.
13. RCOG. Fetal and perinatal pathology. Report of a joint working party. RCOG. 2001. London.
14. Bove KE. Practice guidelines for autopsy pathology: the perinatal and pediatric autopsy. Autopsy Committee of the College of American Pathologists. Arch Pathol Lab Med. 1997;121:368-376.
15. Langston C, Kaplan C, Macpherson T et al. Practice guideline for examination of the placenta: developed by the Placental Pathology Practice Guideline Development Task Force of the College of American Pathologists. Arch Pathol Lab Med. 1997;121:449-476.
16. Kloosterman GJ. On intrauterine growth. The significance of prenatal care. Int J Gynaecol Obstet. 1970;895-912.
17. Brown MA, Lindheimer MD Swiet M de et al. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Association for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy 20, IX-XIV. 2001.
18. Fox H. Pathology of the Placenta. second ed. London: Saunders Company; 1997.
19. Stallmach T, Hebisch G, Meier K, Dudenhausen JW, Vogel M. Rescue by birth: defective placental maturation and late fetal mortality. Obstet Gynecol. 2001;97:505-509.

20. Katzman PJ, Genest DR. Maternal floor infarction and massive perivillous fibrin deposition: histological definitions, association with intrauterine fetal growth restriction, and risk of recurrence. Pediatr Dev Pathol. 2002;5:159-164.

21. Redline RW, Pappin A. Fetal thrombotic vasculopathy: the clinical significance of extensive avascular villi. Hum Pathol. 1995;26:80-85.

22. Gordijn SJ, Korteweg FJ, Erwich JJ et al. A multilayered approach for the analysis of perinatal mortality using different classification systems. Eur J Obstet Gynecol Reprod Biol. 2009.

23. Sebire NJ, Goldin RD, Regan L. Term preeclampsia is associated with minimal histopathological placental features regardless of clinical severity. J Obstet Gynaecol. 2005;25:117-118.

24. Moldenhauer JS, Stanek J, Warshak C, Khoury J, Sibai B. The frequency and severity of placental findings in women with preeclampsia are gestational age dependent. Am J Obstet Gynecol. 2003;189:1173-1177.

25. Zhang P, Schmidt M, Cook L. Maternal vasculopathy and histologic diagnosis of preeclampsia: poor correlation of histologic changes and clinical manifestation. Am J Obstet Gynecol. 2006;194:1050-1056.

26. Ananth CV, Berkowitz GS, Savitz DA, Lapinski RH. Placental abruption and adverse perinatal outcomes. JAMA. 1999;282:1646-1651.

27. Gul A, Cebeci A, Aslan H, Polat I, Ozdemir A, Ceylan Y. Perinatal outcomes in severe preeclampsia-eclampsia with and without HELLP syndrome. Gynecol Obstet Invest. 2005;59:113-118.

28. Khong TY, Chambers HM. Alternative method of sampling placentas for the assessment of uteroplacental vasculature. J Clin Pathol. 1992;45:925-927.

29. Sun CC, Revell VO, Belli AJ, Viscardi RM. Discrepancy in pathologic diagnosis of placental lesions. Arch Pathol Lab Med. 2002;126:706-709.

30. Fuke Y, Aono T, Imai S, Suehara N, Fujita T, Nakayama M. Clinical significance and treatment of massive intervillous fibrin deposition associated with recurrent fetal growth retardation. Gynecol Obstet Invest. 1994;38:5-9.

31. Boog G. Chronic villitis of unknown etiology. Eur J Obstet Gynecol Reprod Biol. 2008;136:9-15.

32. Collins JH. Umbilical cord accidents: human studies. Semin Perinatol. 2002;26:79-82.

Chapter 5

Placental villus immaturity as an important cause of term fetal death

Submitted

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ABSTRACT

Background
Little is known about intrauterine fetal deaths caused by placental villus immaturity.

Objective
Our objective was to describe the prevalence and clinico-pathological associations of intrauterine fetal deaths caused by placental villus immaturity.

Methods
In a prospective study of 1025 couples with singleton intrauterine fetal deaths beyond 20 weeks of gestation we studied all cases beyond 36 weeks of gestation (n=352). The Tulip classification was used for allocation of the cause of death. Based on these causes of death the IUFD's were divided in three groups: villus immaturity, other placental pathology and non-placental pathology.

Results
The overall prevalence of villus immaturity was 23% (81/352). Absolute placental hypoplasia, also a developmental pathology of the placenta, was found twice as often in fetal deaths caused by villus immaturity (43.4%) compared to non-placental causes (19.7%) (p=0.006). Comparable differences were found for relative placental hypoplasia. Oligohydramnios occurred almost twice as often in the group with villus immaturity (23.1%) than in the group with non-placental causes (12.5%, p=0.14). The prevalence of gestational diabetes was 2.5 fold-higher in the villus immaturity group than in the group caused by other placental pathology (13.9% versus 5.5%) (p=0.03) and 10 fold-higher than in the group caused by non-placental pathology (13.9% versus 1.4%) (p=0.005).

Conclusion
Villus immaturity is an important cause of term fetal death and is associated with gestational diabetes and placental hypoplasia.

INTRODUCTION

Over 60% of intrauterine fetal deaths (IUFD) are reported to have a placental cause of death.[1-4] Different placental pathologies occur in different gestational periods of pregnancy. In mid-trimester pregnancy (24-32 weeks of gestation) placental bed pathology, characterized by inadequate spiral artery remodeling and/or spiral artery pathology, causes death in more than half of IUFD. In term pregnancy developmental pathology of the placenta characterized by morphologic abnormalities due to abnormal development, causes IUFD in 40%.[5]

Fetal development and wellbeing are dependent on placental function and maturation. Placental maturation is a gradual process that proceeds throughout pregnancy. During the last months of pregnancy fetal oxygen and nutrient requirements increase. The placenta compensates for the increased needs by an expansion of the maternal-fetal exchange surface, forming the so-called syncytiovascular membranes (SVM) in the tertiary villi. SVM are very thin membranes with a maternal to fetal diffusion distance of only about 3.7μm that allow efficient transport.[6]

Stallmach et al. have described immaturity of the tertiary villi with a reduced number of SVM as a cause of fetal death in their population survey of 17,415 consecutive unselected singleton placentas (beyond 32 weeks of gestation).[7] They concluded that defective maturation of the placenta results in a 70 fold risk of fetal death, but that few affected fetuses actually die. They reported a prevalence of 5.7% including 2.3% associated with fetal death and a tenfold risk of recurrent fetal death.[7] De Laat et al confirmed an association between fetal death and villus immaturity. In their study the odds ratio for fetal death was 132 (95% CI: 13.2-1315) in the presence of a mean number of SVM under the 10th percentile. They found a trend towards the combination of hyper coiling of the umbilical cord and villus immaturity.[8]

Our aim was to describe the prevalence and clinico-pathological associations of term IUFD caused by villus immaturity alone, or villus immaturity in combination with other placental pathologies in our cohort of 1025 IUFD beyond 20 weeks of gestation.

MATERIALS AND METHODS

In 2002 we initiated a Dutch prospective IUFD cohort study in 50 secondary and tertiary referral hospitals, serving a rural as well as an urban population. Inclusion criteria were singleton fetal death diagnosed ante partum (heart beat ceased before labor) after 20 weeks of gestation calculated from the last menstrual period

and confirmed by ultrasonography. Pregnancy terminations and intrapartum fetal deaths were excluded.

The study was approved by the institutional review boards of all participating hospitals. Written informed consent was obtained from all participants. Collected data included medical and obstetric history; maternal and fetal characteristics; and pregnancy and birth details. Our diagnostic workup protocol included: extensive maternal blood tests including full blood count, chemistry and viral serology, and coagulation tests performed by a central laboratory; fetal blood tests including viral serology; microbiological cultures from mother, fetus and placenta; autopsy; placental examination; and cytogenetic analysis.

Autopsy and placental examination were performed by the consulting surgical and perinatal pathologists in the participating hospitals in accordance with guidelines published by the Royal College of Obstetricians and Gynecologists and the Royal College of Pathologists[9] and the College of American Pathologists.[10,11] All examined placentas were studied histopathologically.

Fetal growth percentiles for birth weight by gestational age at time of diagnosis of fetal death were calculated according to the Kloosterman growth charts.[12] Small for gestational age (SGA) was defined as birth weight below the 10^{th} percentile, large for gestational age (LGA) was defined as birth weight > 90^{th} percentile. Definitions for hypertension-related disease (chronic hypertension, pregnancy induced hypertension (PIH), pre-eclampsia, HELLP syndrome and superimposed conditions) were based on recommendations by the International Society for the Study of Hypertension in Pregnancy.[13] Placental hypoplasia was defined as an absolute too low placental weight < 10^{th} percentile (absolute placental hypoplasia) and/or a too low placenta/birth weight ratio (relative placental hypoplasia).[14] Villus immaturity was defined as a placental maturation defect after 36 weeks of gestation with deficient formation of syncytiovascular membranes as interpreted by the consulting pathologist.[5,7,14] The umbilical cord coiling index (UCI) was calculated as the number of coils in the umbilical cord divided by the cord length in meters. Normal range: 0.1-0.3 based on previously published normal values of the UCI.[15-17]

All cases were classified according to the Tulip classification for perinatal mortality (Table 1) by a panel of two consulting obstetricians, one registrar in Obstetrics and Gynecology and one perinatal pathologist for determination of the cause of death.[2] Cause of death was defined as the initial, demonstrable pathophysiological entity initiating the chain of events that had irreversibly led to death. Risk factors such as smoking or hypertension, defined as other known contributing factors to death, were identified. Only one underlying cause of death could be allocated. Subgroups of a placental cause of death were defined as described previously.[2,14]

Villus immaturity

Villus immaturity is a subcategory of placental developmental pathology in the Tulip classification (Table 1).[2,5,14] We evaluated the presence of villus immaturity alone (Tulip: 'placental development') or villus immaturity in combination with other placental pathologies (Tulip: 'placenta not otherwise specified'), as a cause of death in all term IUFD over 36 completed weeks. IUFD was caused either by villus immaturity alone or by villus immaturity in combination with other placental pathology. For comparison of characteristics and clinico-pathological associations we used two groups. The first group consisted of fetal deaths caused by placental pathology other than villus immaturity ('other placental'). The second group consisted of fetal deaths with a non-placental cause of death, including 'unknown' and 'other' causes of death ('non-placental'). Furthermore, maternal conditions that contributed to death, such as hypertension and diabetes, as well as known risk factors for death were selected as possible variables for analysis.

Table 1. Tulip classification, placental categories.

Cause	Subclassification	
1 Congenital anomaly		
2 Placenta	1 Placental bed pathology	
	2 Placental pathology	1 Developmental
		2 Parenchyma
		3 Localisation
	3 Umbilical cord complication	
	4 Not otherwise specified	
3 Prematurity/immaturity		
4 Infection		
5 Other		
6 Unknown		

Statistics

Categorical variables were expressed as counts and percentages and continuous data as median and ranges. Differences between groups for categorical data were evaluated by Fisher exact test or Chi Square test. For continuous variables the Mann-Whitney U test was used. A two-tailed p-value <0.05 was considered to indicate statistical significance. Statistical analyses were performed using SAS software, version 9 (SAS-Institute Inc., Cary, NC, USA).

RESULTS

In our national IUFD study we analyzed 1025 couples and their IUFD. In this cohort 352 IUFD occurred after 36 completed weeks of gestation and were included in our analysis. A placental cause of death was identified in 280 of these 352 cases (80%). The overall prevalence of villus immaturity was 23% (81/352). Twenty-nine percent (81/280) of the placental causes of death represented villus immaturity, either villus immaturity alone or villus immaturity in combination with other placental pathologies. In the case of combined pathologies, placental hypoplasia and fetal thrombotic vasculopathy were most often present (Table 2). The prevalence of IUFD with a placental cause other than villus immaturity was 57% (199/352). In 20% of the IUFD, the cause of death was non-placental (72/352).

Table 2. Villus immaturity and possible combinations with other placental pathologies as a cause of intrauterine fetal deaths.

	n (%)	n(%)[A]
Villus immaturity[C]	39 (48.1%)	
Villus immaturity and combined placental pathology[D]	42 (51.9%)	
Hypoplasia		31 (75.6%)
Fetal thrombotic vasculopathy		15 (36.6%)
Villitis of unknown origin		7 (17.1%)
Abruption		4 (9.8%)
Infarction		4 (9.8%)
Umbilical cord occlusion		4 (9.8%)
Chromosomal		1 (2.4%)
Excessive bleeding		1 (2.4%)
Total	81 (100%)	

[A]Overlap between different forms of pathology exist, therefore the number is not equal to 42 and the percentage exceeds 100. [C]Villus immaturity as classified in the Tulip: developmental pathology. [D]Combined placental pathologies as classified in the Tulip: placental not otherwise specified.

Absolute placental hypoplasia was found twice as often in placentas of fetal deaths caused by villus immaturity (43.4%) than in placentas with non-placental causes (19.7%) (p=0.006). The difference between other placental causes (62.1%) and non placental causes (19.7%) was even higher. (p=0.004) Comparable differences were found for relative placental hypoplasia although statistically no difference was reached between IUFD with villus immaturity and IUFD with other placental causes (Table 3).

Oligohydramnios occurred almost twice as often in deaths caused by villus immaturity (23.1%) than in deaths with a non-placental cause of death (12.5%, p=0.14). The prevalence of oligohydramnios did not differ between villus

Table 3. Clinico-pathological associations of IUFD with villus immaturity as the cause of death, with other placental causes of death and non-placental causes of death.

Cause of death	Villus immaturity n=81	Other placental n=199	p	Non-placental n=72	p
1 Placental characteristics					
Absolute placental[A] hypoplasia	43.4% (76)	62.1% (195)	0.006	19.7% (61)	0.004
Relative placental[A] hypoplasia	66.7% (78)	77.6% (196)	0.067	30.6% (62)	<0.001
Coiling index[A]					
<0.1	29.4% (51)	24.0% (75)	0.15	33. 3% (21)	0.17
0.1-0.3	58.8%	50. 7%		38.1%	
>0.3	11.8%	25. 3%		28.6%	
Umbilical cord insertion[A, C]					
(Para)central	79.2% (72)	80.5% (174)	0.61	79.3% (58)	0.91
Velamentous/marginal	14.0%	15.5%		12.1%	
Not known	6.9%	4.0%		8.6%	
2 Amniotic fluid characteristics					
Oligohydramnios	22.2% (81)	23.1% (199)	1.0	12.5% (72)	0.14
3 Maternal characteristics					
Maternal age					
Median	31.4 (81)	31.4 (199)	0.73	32.0 (72)	0.98
Range	17.5-42.6	21.1-45.2		20.8-38.9	
BMI					
Median	26.8 (64)	25.16 (155)	0.12	25.24 (48)	0.20
Range	20.0-47.8	17.0-46.7		15.8-38.2	
Parity					
Nulliparous	45.7%(81)	56.3% (199)	0.11	55. 6% (72)	0.26
Multiparous	54.3%	43.3%		44.4%	
Diseases					
Pre-existent hypertension	1.2% (81)	2.5% (199)	0.68	0% (72)	1.00
Pregnancy induced hypertension	8.6%	10.6%	0.82	4.2%	0.33
Pre-eclampsia	1.23%	3.0%	0.68	1.4%	1.00
HELLP	0.0%	0.5%	1.00	0.0%	-
Diabetes	2.5%	2.0%	1.00	0.0%	0.50
Gestational diabetes	13.6%	5.5%	0.03	1.4%	0.005
Intoxications[A]					
Smoking	18.5% (79)	23.1% (198)	0.40	18.1% (70)	0.27
Alcohol	3.8% (81)	3.1% (199)	0.72	5.8% (72)	0.71
Other drugs	2.5% (81)	1.5% (199)	0.50	0.0% (72)	0.63
4 Paternal characteristics					
Paternal age					
Median	34.1 (81)	34.4 (199)	0.73	34.7 (72)	0.96
Range	24.0-48.4	22.7-53.3		19. 3-53.1	

Table 3. (cont)

5 Fetal characteristics					
Sex					
Boy	54.3% (81)	51.8% (199)	0.79	55.6% (72)	1.00
Girl	45.7%	48.2%		44.4%	
Fetal weight[B]					
< p10	14.8% (81)	24.1% (199)	0.11	20.8% (72)	0.40
> p90	16.0%	12.1%	0.44	15.3%	0.85

[A]The percentage calculated as percentage of cases over those with known data () [B]Fetal growth of villus immaturity group in comparison to the general population based on the Kloosterman centiles: growth below the 10th percentile: p= 0.22, growth above the 90th percentile: p=0.12. [C]P value for coiling index > 0.3 between villus immaturity and other placental causes: p=0.071 and villus immaturity and non placental causes: p=0.096

immaturity as the cause of fetal death (23.1%) and the other placental causes of fetal death (22.2%, p=1.0).

The umbilical cord coiling-index (UCI) in deaths caused by villus immaturity was statistically not different from the UCI found in deaths with other placental causes of death other than villus immaturity (p=0.15) or non-placental causes of death (p=0.17). The UCI was most often within the normal range in the villus immaturity group (58.8%). The lowest percentage of hyper coiling was found in the group with villus immaturity (11.8%). Unfortunately the UCI could not be determined in 59% of cases (Table 3). Umbilical cord insertion was similar between the groups.

The prevalence of gestational diabetes was 2.5 fold-higher in the villus immaturity group than in the group caused by other placental pathology (13.9% versus 5.5%) (p=0.029) and 10 fold- higher than in the group caused by non-placental pathology (13.9% versus 1.4%) (p=0.005). Other risk factors and maternal conditions, including Body Mass Index (BMI), did not differ between the groups (Table 3). Paternal age was comparable in all groups.

In IUFD caused by villus immaturity fetuses were SGA in 14.8% and LGA in 16%, which is statistically not different compared to the general population (respectively p=0.22 and p=0.12). In almost a quarter of IUFD with a placental cause of death other than villus immaturity and in 20.8% of the IUFD with a non placental cause of death fetuses were SGA, which was statistically not different from the prevalence of SGA in fetuses with villus immaturity as the cause of death. The prevalence of LGA was neither different between the groups (Table 3). Fetal gender was equally represented in all groups (Table 3).

Only one case of villus immaturity coincided with an abnormal fetal chromosomal pattern: 45X/46XY (Table 2).

DISCUSSION

Placental villus immaturity is an important cause of term IUFD. Villus immaturity was associated with gestational diabetes and also associated with placental hypoplasia in comparison to non placental causes.

In our study we primarily focused on IUFD, while others initiated their analysis from a placental point of view.[7,8] Differences in study groups might explain the much higher prevalence of villus immaturity in our IUFD cohort than the previously reported prevalences.[7,18] Two types of villus immaturity at term have been described.[19] In term placentas scattered small groups of immature chorionic villi can be seen in up to 97% of uncomplicated pregnancies. These immature villi are freshly formed and arise directly from the stem villi.[18,20,21] Groups of these immature villi are found in areas of placental growth and are not indicative of placental pathology. In the other pattern almost all chorionic villi are markedly immature for the duration of pregnancy with inadequate formation of SVM due to abnormal developmental processes, which is the pattern we have focused on.[19] However, in the international literature no uniform definition for villus immaturity has been established yet. Some base their definition of villus immaturity on the percentage of villi with SVM or on the number of SVM per terminal villus or per histological sample.[7,8,18,22] Data on the normal quantity of SVM are limited and the mean numbers differ.[7,8,18,19,22] Others use abnormalities in morphology and angiogenesis of the villi to define villus immaturity.[6,18,19,23,24] Akin studies on the nosology and reproducibility of placental reaction patterns[25-27] further studies are needed to review, define, and test the reproducibility of diagnostic criteria for villus immaturity.

Gestational diabetes and pre-existent diabetes have been associated with villus immaturity.[18,19,22-24,28-30] Calderon et al. reported that the size and number of terminal villi as well as villus total area in diabetes were similar to their control group. However, total and mean villus vessel surfaces were smaller in diabetes, resulting in a lower capillarization index.[28] Evers et al also reported an increase in villus immaturity in placentas of diabetic mothers. They found that the appropriate for gestation (AGA) babies of diabetic women had a relatively high placental weight, which they suggested as possibly compensating for villus immaturity as a protection against hypoxemia. Their LGA babies had a relatively lower placental weight, which may explain the increased incidence of fetal death in that category.[24] Also in our study villus immaturity was associated with placental hypoplasia and gestational diabetes. The association of villus immaturity and gestational diabetes, but not with pre-existent diabetes between groups is remarkable. The reason for this difference is speculative, but may be related to differences in glycemic control.

Placental pathology can lead to decreased placental function, resulting in fetal growth restriction and oligohydramnios. In these cases the mode of fetal death is chronic. Villus immaturity is a parenchymal disease characterized by inadequate development of SVM in tertiary villi. The absence of SVM is thought to cause placental dysfunction in a period of pregnancy when demands on placental function are increased. Signs of hypoxemia in these cases of late fetal death support placental dysfunction as a mechanism of death in these cases.[8] In deaths caused by villus immaturity the mode of death is unknown and can only be speculated on. In appropriately grown fetuses a chronic mode of death seems less likely. A sub-acute mode of death is suggested by a trend towards increased prevalence of oligohydramnios in IUFD with villus immaturity as a sign of redistribution of fetal blood to vital organs at expense of renal blood flow. In the fetal deaths caused by villus immaturity the prevalence of SGA was found to be 50% higher than in the general population. Based on this trend towards a lower fetal weight, one might suggest that at least in some of the SGA fetuses the mode of death is chronic as these fetuses might have grown less than their potential and are growth restricted. Use of customized growth charts to detect growth restricted fetuses[31,32] and correlation of clinical signs and symptoms with data on the mode of death obtained from autopsy studies could corroborate the mode of death further.[33]

In conclusion, placental villus immaturity is an important cause of term IUFD. In pregnancy villus immaturity may only present with few clinical signs and symptoms which hinders intervention to prevent death. Recurrent disease has been described and only in these cases pregnancy may be rescued by birth.[7]

REFERENCES

1. Galan-Roosen AE, Kuijpers JC, van der Straaten PJ, Merkus JM. Evaluation of 239 cases of perinatal death using a fundamental classification system. Eur J Obstet Gynecol Reprod Biol. 2002;103:37-42.
2. Korteweg FJ, Gordijn SJ, Timmer A et al. The Tulip classification of perinatal mortality: introduction and multidisciplinary inter-rater agreement. BJOG. 2006;113:393-401.
3. Incerpi MH, Miller DA, Samadi R, Settlage RH, Goodwin TM. Stillbirth evaluation: what tests are needed? Am J Obstet Gynecol. 1998;178:1121-1125.
4. Rayburn W, Sander C, Barr M, Jr., Rygiel R. The stillborn fetus: placental histologic examination in determining a cause. Obstet Gynecol. 1985;65:637-641.
5. Korteweg FJ, Erwich JJHM, Holm JP et al. Diverse placental pathologies as the main causes of fetal death. Obstet Gynecol. Obstet Gynecol. 2009;114:809-817.
6. Benirschke K, Kaufmann P. Pathology of the Human Placenta. 4th edition. New York: Springer-Verlag; 2000.
7. Stallmach T, Hebisch G, Meier K, Dudenhausen JW, Vogel M. Rescue by birth: defective placental maturation and late fetal mortality. Obstet Gynecol. 2001;97:505-509.
8. de Laat MW, van der Meij JJ, Visser GH, Franx A, Nikkels PG. Hypercoiling of the umbilical cord and placental maturation defect: associated pathology? Pediatr Dev Pathol. 2007;10:293-299.
9. RCOG. Fetal and perinatal pathology. Report of a joint working party. RCOG. 2001. London.
10. Bove KE. Practice guidelines for autopsy pathology: the perinatal and pediatric autopsy. Autopsy Committee of the College of American Pathologists. Arch Pathol Lab Med. 1997;121:368-376.
11. Langston C, Kaplan C, Macpherson T et al. Practice guideline for examination of the placenta: developed by the Placental Pathology Practice Guideline Development Task Force of the College of American Pathologists. Arch Pathol Lab Med. 1997;121:449-476.
12. Kloosterman GJ. Intrauterine growth and intrauterine growth curves. Ned Tijdschr Verloskd Gynaecol. 1969;69:349-365.
13. Brown MA, Lindheimer MD, Swiet M de et al. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Association for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy 20, IX-XIV. 2001.
14. Korteweg FJ, Gordijn SJ, Timmer A, Holm JP, Ravise JM, Erwich JJ. A placental cause of intra-uterine fetal death depends on the perinatal mortality classification system used. Placenta. 2008;29:71-80.
15. Machin GA, Ackerman J, Gilbert-Barness E. Abnormal umbilical cord coiling is associated with adverse perinatal outcomes. Pediatr Dev Pathol. 2000;3:462-471.
16. van Dijk CC, Franx A, de Laat MW, Bruinse HW, Visser GH, Nikkels PG. The umbilical coiling index in normal pregnancy. J Matern Fetal Neonatal Med. 2002;11:280-283.
17. Strong TH, Jr., Jarles DL, Vega JS, Feldman DB. The umbilical coiling index. Am J Obstet Gynecol. 1994;170:29-32.
18. Fox H. Villous immaturity in the term placenta. Obstet Gynecol. 1968;31:9-12.
19. Fox H, Sebire N. Histological Abnormalities of the Placenta. Pathology of the Placenta. 3rd edition. Philadelphia: Saunders Elsevier; 2007:95-146.

20. Stallmach T, Hebisch G. Placental pathology: its impact on explaining prenatal and perinatal death. Virchows Arch. 2004;445:9-16.

21. Bleyl U, Stefek E. On the morphology and diagnostic evaluation of loose immature villi in mature human placenta. Beitr Pathol Anat. 1965;131:162-183.

22. Faye Petersen OM, Heller DS, Joshi VV. Histologic lesions of the placenta. Handbook of Placental Pathology. 2nd edition. London, New York: Taylor & Francis; 2006:53-79.

23. Daskalakis G, Marinopoulos S, Krielesi V et al. Placental pathology in women with gestational diabetes. Acta Obstet Gynecol Scand. 2008;87:403-407.

24. Evers IM, Nikkels PG, Sikkema JM, Visser GH. Placental pathology in women with type 1 diabetes and in a control group with normal and large-for-gestational-age infants. Placenta. 2003;24:819-825.

25. Redline RW, Ariel I, Baergen RN et al. Fetal vascular obstructive lesions: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2004;7:443-452.

26. Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2003;6:435-448.

27. Redline RW, Boyd T, Campbell V et al. Maternal vascular underperfusion: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2004;7:237-249.

28. Calderon IM, Damasceno DC, Amorin RL, Costa RA, Brasil MA, Rudge MV. Morphometric study of placental villi and vessels in women with mild hyperglycemia or gestational or overt diabetes. Diabetes Res Clin Pract. 2007;78:65-71.

29. Laurini RN, Visser GH, van BE, Schoots CJ. Morphological findings in placentae of insulin-dependent diabetic patients treated with continuous subcutaneous insulin infusion (CSII). Placenta. 1987;8:153-165.

30. Teasdale F. Histomorphometry of the placenta of the diabetic women: class A diabetes mellitus. Placenta. 1981;2:241-251.

31. Gardosi J, Chang A, Kalyan B, Sahota D, Symonds EM. Customised antenatal growth charts. Lancet. 1992;339:283-287.

32. Gardosi J. The application of individualised fetal growth curves. J Perinat Med. 1998;26:333-338.

33. Becker MJ, Becker AE. Fat distribution in the adrenal cortex as an indication of the mode of intrauterine death. Hum Pathol. 1976;7:495-504.

Chapter 6

Cytogenetic analysis after evaluation of 750 fetal deaths; proposal for diagnostic workup

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ABSTRACT

Objective

To estimate success rates for cytogenetic analysis in different tissues after intrauterine fetal death, and study selection criteria and value of cytogenetic testing in determining cause of death.

Methods

Cytogenetic analyses and the value of this test in determining cause by a multidisciplinary panel were studied in 750 fetal deaths. Morphological abnormalities, small for gestational age (SGA), advanced maternal age (older than 35 years) and maceration were studied as selection criteria.

Results

Chromosomal abnormalities were observed in 13% of fetal deaths. Cytogenetic success rates were significantly higher for invasive testing (85%) than for post partum tissue analysis (28%, $p<0.001$). There were more abnormal chromosomes (38%) in fetal deaths with morphologic abnormalities than in those without (5%, $p<0.001$). This was not observed for SGA (16% compared with 9.2%, $p=0.22$) or for advanced maternal age (16.7% compared with 12.0%, $p=0.37$). The posterior probability of a chromosomal abnormality in the absence of morphological abnormalities was still 4.6%. Cytogenetic analysis was successful in 35% of severely macerated fetuses. We do not advise using these selection criteria because the failure rate was high on postpartum tissues. Cytogenetic analysis was valuable in determining the cause in 19% of fetal deaths.

Conclusion

Parents should be counseled on aspects of cytogenetic analysis after fetal death. We advise performing non-selective invasive testing after fetal death and before labor for all fetal deaths.

INTRODUCTION

The cause of death remains unexplained in about two-thirds of stillbirths.[1-5] It is useful to identify a cause of death: it helps parents in their mourning process, it may determine the recurrence risk, aid counseling for future pregnancies, siblings and families, enables comparison of national and international health care and aids prevention.[6-8]

One of the causes of stillbirth is a chromosomal anomaly. The incidence of these anomalies, which are detectable through karyotyping, has been reported to be 6-12% in a random selection of stillborns.[3,9,10] There are no international uniform protocols for cytogenetic analysis available based on evidence of stillbirths.[2,10] Some studies recommend cytogenetic analysis for all fetal deaths,[9,10] whereas others advise testing for a selected population, mainly due to the substantial costs. This selection would cover deaths with, e.g., congenital anomalies, fetal growth restriction, nonmacerated fetuses, advanced maternal age, or recurrent pregnancy loss.[5,10-15] In several European countries it is common practice that cytogenetic analysis is only indicated (funded by insurance companies) if congenital anomalies are present either at ultrasound or at birth.[16] Gynecologists (both qualified and in training) or midwives are often responsible for evaluating these morphologic abnormalities at birth and also, therefore, for the decision to perform cytogenetic analysis.

Results of tissue cultures after intrauterine fetal death are often disappointing. Overall, successful tissue culture followed by karyotyping has been reported in 41% of intrauterine fetal deaths but depends on the type of tissue examined. Karyotyping in this group was successful in 18% of intrauterine fetal deaths for skin biopsies, in 7% of other fetal tissues, and in up to 71% of extrafetal tissues such as placenta.[17] To increase the rate of successful tests, amniocentesis or chorionic villi sampling (CVS) before induction of labor is advised.[18-20] However, good alternatives are needed if this is not feasible due to objection by parents, inexperience of the gynecologist, diminished amniotic fluid, or logistic problems.[2] There is no consensus on which tissue type is best for cytogenetic testing post partum. The aim of our study was to estimate success rates of cytogenetic analysis in different tissue types after intrauterine fetal death, to study selection criteria for cytogenetic analysis, and the value of cytogenetic testing in determining the cause of death. Our goal was to design a flow chart to help determine which intrauterine fetal deaths should have cytogenetic analysis performed and to guide counseling of the parents through the decision on whether to allow this test.

MATERIALS AND METHODS

In 2002 we initiated a national Dutch study on intrauterine fetal death at the University Medical Centre Groningen, with 50 participating hospitals located throughout the Netherlands. Inclusion criteria for the study were singleton intrauterine fetal deaths diagnosed antepartum (heart beat ceased before start of labor) after 20 weeks of gestation and confirmed by ultrasonography. Pregnancy terminations were excluded. The University Medical Centre Groningen Institutional Review Board Committee determined that this study was exempt. Clinical data were collected after informed consent was obtained. For each intrauterine fetal death, a case record form was filled in and a standard diagnostic workup protocol was followed. There is no national diagnostic work-up guideline for intrauterine fetal death. Our diagnostic study protocol was therefore based on local protocols currently used in different Dutch hospitals.

Diagnostic protocol

Patient data included medical and obstetric history, maternal characteristics, fetal characteristics, pregnancy details, and obstetric discharge letters. The diagnostic test results included maternal and fetal blood tests; maternal and fetal viral serology; microbiological cultures from mother, fetus and placenta; autopsy; placental examination; and cytogenetic analysis. Parental consent was obtained for all the diagnostic work.

In the Dutch genetic guidelines, cytogenetic analysis is indicated in the event of stillbirth after 16 weeks of gestation if fetal congenital anomalies are present at ultrasound or at birth. There is no national consensus on which tissue is best to use. Our study protocol prescribed cytogenetic evaluation of all intrauterine fetal deaths either by invasive testing (amniocentesis or CVS) before induction of labor or by postpartum tissue testing of fetus (fetal blood, fascia lata, pericardium, cartilage, or skin), umbilical cord or placenta. In some cases, amniocentesis, CVS or preimplantation diagnostics had already been performed during the ongoing pregnancy and before fetal death for various reasons. In most cases, cytogenetic analysis was performed after intrauterine fetal death was diagnosed, but in a small group of cases both types of cytogenetic analysis were performed. Multiple tissues were analyzed in some cases.

The biopsies were collected in a sterile pot containing saline solution or culture medium and transported to the laboratory. Cytogenetic analysis was performed in local, specialized genetic laboratories following standard laboratory procedures consistent with the guidelines of the Dutch Association of Clinical Cytogeneticists. Mostly, chromosomal analysis was performed in fibroblast cultures. In some cases, additional molecular cytogenetic analyses, such as fluorescent in situ

hybridization, multiplex ligation-dependent probe amplification, or comparative genomic hybridization were done if fibroblast cultures failed.

Autopsy and placental examination were performed by local pathologists in the participating hospitals. Pathologists were urged to follow the study guidelines for autopsy and placental examination based on those published by the Royal College of Obstetricians and Gynecologists and the Royal College of Pathologists[21], and the College of American Pathologists.[22,23]

Morphologic abnormalities determined at birth, small for gestational age (SGA), maternal age 35 or more years and maceration were studied as possible selection criteria for cytogenetic analysis. Morphologic abnormalities of the fetus at birth, as determined by the gynecologist, gynecologist-in-training or midwife, were all adjudicated by an independent perinatal clinical geneticist as common variant, minor or major abnormalities, based on the phenotypic abnormality classification by Merks et al.[24,25] The geneticist did not have access to the cytogenetic or autopsy results. We added hydrops as a major anomaly and single umbilical artery as a minor anomaly. Fetal growth percentiles for birth weight by gestational age at time of diagnosis of intrauterine fetal death were calculated according to the Kloosterman growth charts,[26] which start at 25 weeks of gestation. Small for gestational age was defined as a birth weight at less than the 10th percentile. The stage of maceration was classified according to Wigglesworth.[27]

Classification of cause of death

Panel classification sessions were set up for determining cause of death and the value of cytogenetic analysis in this determination. Procedures were agreed upon in advance and the Tulip classification for cause of death was used.[28] The panel consisted of two obstetricians, an obstetric resident, and a pediatric pathologist. All panel members first prepared each case individually using the patient information records, then panel discussions were held and a consensus reached on cause of death and the value of the diagnostic test. No other information sources were consulted. The value of cytogenetic analysis in determining cause of death was assessed under four headings as 'establishing cause of death' (abnormal results of cytogenetic analysis established a cause), 'excluding cause of death' (the results of cytogenetic analysis excluded this cause in an intrauterine fetal death with a suspected chromosomal abnormality), 'missing for determination of cause of death' (in an intrauterine fetal death with a suspected chromosomal abnormality, the results of cytogenetic analysis were missing), or 'not valuable in determining cause of death' (cytogenetic analysis was not regarded as valuable in this process).

Statistics

Continuous variables were expressed as median values, and ranges and categorical data were expressed as counts and percentages. Differences between groups for categorical data were evaluated by using the Fisher exact test or chi square test. In addition, exact 95% confidence intervals were presented when applicable. A two-tailed p-value <0.05 was considered to indicate statistical significance. Statistical analyses were performed using SAS software, version 9.1 (SAS-Institute Inc., Cary, NC, USA).

RESULTS

A total of 750 intrauterine fetal deaths were studied during a four-year period from 2002 to 2006. The participating hospitals started including intrauterine fetal deaths at different points in time and the inclusion rates per hospital differed. An investigation into these rates yielded an average inclusion rate of 75% of intrauterine fetal deaths that met our selection criteria. The reasons for exclusion were: informed consent denied, a language barrier, logistic problems, and a doctor's decision to exclude an intrauterine fetal death in the case of a "known" cause of death at birth (placental abruption, known chromosomal abnormalities and, major congenital anomalies).

The median age of the mothers was 31 years (range 17-46 years), and the median gestational age at determination of intrauterine fetal death was 31 weeks and 4 days (range 20-42 weeks and 1 day). The median fetal weight was 1470 g (range 12-4630 g). Oligohydramnions or anhydramnions was present at diagnosis of intrauterine fetal death in 188 cases (25.1%). Significantly more intrauterine fetal deaths were males (n=408) than females (n=339; $p<0.01$). In three cases, the fetal sex could not be determined by physical, cytogenetic or pathological examination. Autopsy was performed on 525 fetuses (70.0%).

Cytogenetic analysis was performed for 508 intrauterine fetal deaths (67.7%) and a successful result was obtained in 246 deaths (48.4%). Amniocentesis and/or CVS or preimplantation diagnostics were performed during 74 (14.6%) ongoing pregnancies before intrauterine fetal death. The overall success rate during ongoing pregnancy was 100%. Cytogenetic analysis was performed after death in 453 intrauterine fetal deaths (cultures and molecular cytogenetic analysis) and was successful in 39.7%. In 19 intrauterine fetal deaths, tissue samples were taken during ongoing pregnancy as well as after death. The success rates of cytogenetic analyses in relation to tissue type are presented in Table 1. The highest success rates after death were seen for invasive samples (84.6%), CVS (two cases – both successful) or amniocentesis (84.0%), and for intrauterine fetal deaths for which

invasive sampling was performed in combination with fetal, placental, or umbilical cord biopsies (85.7%). Invasive cytogenetic testing after death yielded significantly more successful cytogenetic results than postpartum analyses ($p<0.001$). Cytogenetic success rates on tissues taken post partum varied between 32.1% for umbilical cord and 0% for pericardium. After excluding the pericardium from this group, umbilical cord samples (32.1%, 95% confidence interval [CI] 25.6-39.2) were no more successful than fetal blood and "other tissue types" (20.0%, 95% CI 8.4-36.9; $p=0.17$). The group "other tissue types" included a placenta biopsy (n=1), skin biopsies (n=15), and unknown tissue type (n=10). Additional molecular cytogenetic analysis after failure of fibroblast cultures was performed in six cases during ongoing pregnancy and in 39 cases postpartum. These comprised 36 fluorescent in situ hybridization (4 abnormal), 8 multiplex ligation-dependent probe amplification (1 abnormal), and 1 comparative genomic hybridization.

The prevalence (prior probability) of a chromosomal abnormality in the 246 intrauterine fetal deaths for which a successful cytogenetic result was obtained was 13.0% (95% CI 9.1-17.9). The 32 chromosomal abnormalities in relation to the moment cytogenetic analysis was performed, tissue type tested, and cause of death are shown in Table 2. Trisomy 21 was established in 10 of 32 (31%), trisomy 18 in 7 of 32 (22%), monosomy (45, X) in 7 of 32 (22%), trisomy 13 in 2 of 32 (6%) and other chromosomal abnormalities in 6 of 32 (19%). Fewer than one half of the chromosomal abnormalities (40.6%) were determined in second trimester intrauterine fetal deaths.

The outcome of cytogenetic analysis in relation to the morphologic abnormalities seen at birth by the physician attending the delivery and classified afterwards by a perinatal geneticist are presented in Table 3. Of the 246 chromosomal results, 180 were determined after death. In this latter group of 180 intrauterine fetal deaths with morphologic abnormalities seen at birth, there were significantly more chromosomal abnormalities (38.0%) than in the group without morphologic abnormalities (4.6%, $p<0.001$). In 19 of the 25 (76.0%, 95% CI 54.9-90.6) intrauterine fetal deaths with a chromosomal abnormality (in this group of 180 deaths), morphologic abnormalities were seen at birth (sensitivity), whereas in the group with normal chromosomal results (n=155), there were no morphologic abnormalities in 124 intrauterine fetal deaths (80.0%, 95% CI 72.8-86.0; specificity). Overall, in 143 out of 180 cases (79.4%, 95% CI 72.8-85.1) morphology matched with the chromosomal results. The absence of morphologic abnormalities for a fetus at birth had a negative predictive value of normal chromosomes of 95.4%, whereas the posterior probability of a chromosomal abnormality was 4.6% (95% CI 1.7-9.8). In contrast, the prior probability of a chromosomal abnormality in our cohort was 13%. The presence of morphologic abnormalities had a 38.0% (95% CI 24.7-52.8) positive predictive value of a chromosomal abnormality.

Table 1. Success rates of cytogenetic analysis in 750 intrauterine fetal deaths in different tissue types: prior to intrauterine fetal death (during ongoing pregnancy) or after diagnosis of intrauterine fetal death

		Successful cytogenetic analysis		
			Culture	
Tissue type	**Total**	**Total**	**Karyotype Normal**	**Karyotype Abnormal**
Prior to IUFD during ongoing pregnancy				
Chorion villus	14 (18.9)	14 (100)	13 (92.9)	1 (7.1)
Amniotic fluid	53 (71.6)	53 (100)	41 (77.35)	6 (11.3)
Chorion villus and amniotic fluid	6 (8.1)	6 (100)	6 (100)	-
Preimplantation diagnostics	1 (1.4)	1 (100)	1 (100)	-
Total	74 (100)	74 (100)	61 (82.4)	7 (9.5)
After diagnosis IUFD				
Chorion villus	2 (0.4)	2 (100)	1 (50.0)	1 (50.0)
Amniotic fluid	75 (16.6)	63 (84.0)	54 (72.0)	9 (12.0)
Postpartum				
Umbilical cord	193 (42.6)	62 (32.1)	40 (20.7)	6 (3.1)
Fascia lata biopsy	43 (9.5)	12 (29.9)	4 (9.3)	1 (2.3)
Cartilage	33 (7.3)	8 (24.2)	3 (9.1)	1 (3.0)
Fetal blood	9 (2.0)	2 (22.2)	2 (22.2)	-
Pericardium	3 (0.7)	0 (0)	-	-
Tissue other	26 (5.7)	5 (19.2)	1 (3.8)	1 (3.8)
Tissue multiple	55 (12.1)	14(25.4)	7 (12.7)	-
Tissue multiple including invasive	14 (3.1)	12 (85.7)	9 (64.3)	1 (7.1)
Total	453 (100)	180 (39.7)	121 (26.7)	20 (4.4)
No cytogenetic analysis	242			
Total	769†			

IUFD: intrauterine fetal death. Data are n (%). * Fluorescent in situ hybridization, multiplex ligation-dependent probe amplification or comparative genomic hybridization. †For 19 IUFDs cytogenetic analysis was performed before IUFD during ongoing pregnancy, as well a safter diagnosis of IUFD.

The outcome of chromosomal analysis in relation to fetal birth weight is presented in Table 4. In the group of SGA fetuses with successful chromosomal analysis, we did not observe a statistically significant difference in deaths with a chromosomal abnormality (15.5%) versus non-SGA (9.2%; p=0.22). Nine of 21 intrauterine fetal deaths, (42.9%, 95% CI 21.8-6.0; sensitivity) with a chromosomal abnormality were SGA. Whereas in the group with normal chromosomes (n=167), 118 intrauterine fetal deaths were non-SGA (70.7%, 95% CI 63.1-77.4; specificity). The negative predictive value of non-SGA for normal chromosomes at birth was 90.8%. The posterior probability of a chromosomal abnormality in non-SGA was therefore 9.2% (95% CI 4.9-15.6). The positive predictive value of SGA for a chromosomal abnormality was 15.5% (95% CI 7.4-27.4). Small for gestational age was not associated with a higher failure of cytogenetic analysis (58.9% 95% CI

	Molecular cytogenetic analysis*		Unsuccessful cytogenetic analysis
	Normal	Abnormal	
	-	-	-
	6 (11.3)	-	-
	-	-	-
	-	-	-
	6 (8.1)		-
	-	-	-
	-	-	12 (16.0)
	14 (7.3)	2 (1.0)	131 (67.9)
	7 (16.3)	-	31 (72.1)
	4 (12.1)	-	25 (75.8)
	-	-	7 (77.8)
	-	-	3 (100)
	2 (7.8)	1 (3.8)	21 (80.8)
	5 (9.1)	2 (3.6)	41 (74.6)
	2 (14.3)	-	2 (14.3)
	34 (7.5)	5 (1.1)	273 (60.3)

50.3-76.1 compared with 50.4% (95% CI 44.2-56.6; p=0.12). None of the fetuses with a chromosomal abnormality were reported to be hydropic. For 58 intrauterine fetal deaths, a birth weight percentile could not be calculated. Fifty-seven of these intrauterine fetal deaths were less than 25 weeks of gestation.

The outcome of chromosomal analysis in relation to maternal age is presented in Table 5. Women older than 35 years of age with intrauterine fetal deaths with successful chromosomal analysis did not have significantly more deaths with a chromosomal abnormality (16.7%) than women of 35 years or younger (12.0%, p=0.37). Nine of 32 (28.1%, 95% CI 13.8-46.8; sensitivity) women with an intrauterine fetal death with a chromosomal abnormality were older than 35 years of age, whereas in the group with normal chromosomes (n=214), 169 women (79.0%, 95% CI 72.9-84.2; specificity) were35 years of age or younger. The negative predictive value of maternal age 35 years or younger for normal chromosomes was 88.0%. The posterior probability of a chromosomal abnormality for these

Table 2. The moment cytogenetic analysis was performed, tissue type and cause of death in 32 intrauterine fetal deaths found to have chromosomal abnormalities

Chromosomal abnormality	Moment, tissue type	Cause of death*
47, XX +21	DOP†, amniocentesis	1.1.1
47, XY, +21	DOP, amniocentesis	1.1.1
47, XY, +21	DOP, amniocentesis	1.1.1
47, XX,+ 21	PP‡, umbilical cord	1.1.1
47, XY, +21	AD§ , CVS‖	1.1.1
47, XY, +21	PP, umbilical cord	1.1.1
47, XY, +21	PP, cartilage	2.1
47, XY, +21	AD, amniocentesis	1.1.1
Trisomy 21	PP, various tissues, MLPA	1.1.1
Nuc ish (mos?) (LSI 21X3)[22]/(LSI 21X2)[26]	PP, various tissues, FISH	1.1.1
47, XX, +18	DOP, amniocentesis	1.1.1
47, XX, +18	DOP, CVS	1.1.1
47, XX, +18	AD, amniocentesis	1.1.1
47, XY, +18	AD, amniocentesis	1.1.1
47, XY, +18	PP, tissue other	1.1.1
47, XY, +18	AD, amniocentesis	1.1.1
Trisomy 18	PP, umbilical cord, FISH	1.1.1
45, X	DOP, amniocentesis	1.1.1
45, X	PP, umbilical cord	1.1.1
45, X	PP, umbilical cord	1.1.1
45, X	AD, amniocentesis	1.1.1
45, X	AD, amniocentesis	1.1.1
45, X	PP, various tissues	1.1.1
45, X/46, XY	PP, umbilical cord	1.1.1
47, XX, +13	PP, fascia lata biopsy	1.1.1
Trisomy 13	PP, tissue other, FISH	1.1.1
46, XY, r(13)	DOP, amniocentesis	1.1.2
Triploidy	PP, umbilical cord, FISH	1.1.1
46, XX, ish del (22) (q11.2 q11.2)	AD, amniocentesis	2.2.2
46, XX, t(1:16)(q43;p12)	AD, amniocentesis	2.1
92, XXXX in 9 cells	PP, umbilical cord	2.2.1
Mos 47, XX+i(1)(q10)/46, XX	AD, amniocentesis	1.10

*Cause of death classified according to the Tulip classification28, 1.1.1: congenital anomaly: chromosomal defect; Numerical, 2.1: placental bed pathology, 2.2.2 placental pathology; parenchyma, 2.2.1 placental pathology; development, 1.10 congenital anomaly: neoplasm, †DOP: during ongoing pregnancy, ‡PP: postpartum, §AD: after diagnosis IUFD, ‖CVS: chorionic villus sampling, FISH: fluorescent in situ hybridization, MLPA: multiplex ligation-dependent probe amplification

women was therefore 12.0% (95% CI 7.8-17.4). The positive predictive value of maternal age older than 35 years for a chromosomal abnormality was 16.7% (95% CI 7.9-29.3).

Table 3. Outcome of cytogenetic analysis in relation to morphologic abnormalities

Cytogenetic analysis	Morphologic abnormalities seen at birth by physician		Morphologic abnormalities[24,25] as classified by geneticist afterward			
			None	Minor	Major	Both
Prior to IUFD DOP*	74					
Normal chromosomes	67	27 (40.3)	49 (73.0)	6 (9.0)	6 (9.0)	6 (9.0)
Chromosomal abnormality	7					
47 + 18	2	2 (100)	-	-	2 (100)	-
47 + 21	3	2 (66.7)	1 (33.3)	2 (66.7)	-	-
45, X	1	1 (100)	-	-	1 (100)	-
Other	1	1 (100)	-	-	1 (100)	-
After diagnosis of IUFD	453					
Normal chromosomes	155	31 (20.0)	131 (84.5)	9 (5.8)	12 (7.8)	3 (1.9)
Chromosomal abnormality	25					
45, X	6	6 (100)	-	-	4 (66.7)	2 (33.3)
47 + 13	2	2 (100)	-	-	1 (50.0)	1 (50.0)
47 + 18	5	4 (80.0)	1 (20.0)	1 (20.0)	-	3 (60.0)
47 + 21	6	3 (50.0)	3 (50.0)	1 (16.7)	1 (16.7)	1 (16.7)
Other	6	4 (66.7)	2 (33.3)	1 (16.7)	1 (16.7)	2 (33.3)
Unsuccessful cytogenetic analysis	273	69 (25.3)	229 (83.9)	17 (6.2)	17 (6.2)	10 (3.7)
No cytogenetic analysis	242	26 (10.7)	222 (91.7)	9 (3.7)	7 (2.9)	4 (1.7)
Total	769†	178 (23.1)	638 (83.0)	46 (6.0)	53 (6.9)	32 (4.1)

IUFD: intrauterine fetal death. *DOP: during ongoing pregnancy. Data are n or n (%). †For 19 IUFDs cytogenetic analysis was performed before IUFD during ongoing pregnancy, as well as after diagnosis of IUFD.

The outcome of successful cytogenetic analysis after death (normal and abnormal chromosomes) versus the group in which this was unsuccessful was studied in relation to maceration stage. Maceration stage was unknown for 16 intrauterine fetal deaths. Success rates in intrauterine fetal deaths without maceration was significantly higher 58.5% (95% CI 45.6-70.6) than in intrauterine fetal deaths with maceration (36.6, 95% CI 31.7-41.7; p=0.001). There was no difference in success rates between mildly (43.9%, 95% CI 34.6-53.0), moderately (32.2%, 95% CI 24.9-40.3) or severely macerated fetuses (34.9, 95% CI 25.9-44.8; p=0.14).

Overall, in our cohort of 750 intrauterine fetal deaths, cytogenetic analysis was valuable for determining the cause of death in 18.7% (n=140) of cases. In 2.8% (n=21) of cases the cytogenetic analysis established cause of death, in 7.2% (n=54) the cause of death could be excluded, and in 8.7% (n=65) the panel missed the results of this test for determining cause of death. In 81.3% (n=610) cases, the cytogenetic analysis was not valuable for determining the cause of death.

Table 4. Cytogenetic analysis in relation to fetal birth weight

Cytogenetic analysis		Birth weight percentile			Unknown (<25 weeks)
		<10th	10-90th	>90th	
Total successful	246	58 (30.9)	104 (55.3)	26 (13.8)	58
Chromosomal abnormality	32	9/58 (15.5)	11/104 (10.6)	1/26 (3.8)	11
Normal chromosomes	214	49/58 (84.5)	93/104 (89.4)	25/26 (96.2)	47*
Unsuccessful cytogenetic analysis	262	83 (38.6)	110 (51.2)	22 (10.2)	47
No cytogenetic analysis	242	65 (31.5)	127 (61.7)	14 (6.8)	36
Total	750	206 (33.8)	341 (56.0)	62 (10.2)	141

Data are n, n (%), or n/N (%), *The fetal weight was unknown for 1 IUFD 25 or more weeks of gestation.

Table 5. Cytogenetic analysis in relation to maternal age

Cytogenetic analysis		Maternal age in years			
		<26	26-30	31-35	>35
Total successful	246	41 (16.7)	66 (26.8)	85 (34.6)	54 (21.9)
Chromosomal abnormality	32	8/41 (19.5)	6/66 (9.1)	9/85 (10.6)	9/45 (20.0)
Normal chromosomes	214	33 (15.4)	60 (28.1)	76 (35.5)	45 (21.0)
Unsuccessful cytogenetic analysis	262	53 (20.2)	86 (32.8)	88 (33.6)	35 (13.4)
No cytogenetic analysis	242	52 (21.5)	75 (31.0)	79 (32.6)	36 (14.9)
Total	750	146 (19.4)	227 (30.3)	252 (33.6)	125 (16.7)

Data are n, n (%), or n/N (%).

DISCUSSION

Invasive cytogenetic testing is recommended for all intrauterine fetal deaths before induction of labor because this had the highest success rates. Fetal deaths with morphologic abnormalities determined after birth accounted for most of the chromosomal abnormalities. However, the posterior probability of a chromosomal abnormality in the absence of morphologic abnormalities was still 4.6%. Small for gestational age, advanced maternal age, and maceration stage were not adequate selection criteria for cytogenetic analysis. Cytogenetic analysis post partum in a selected group of intrauterine fetal deaths was not effective, because almost 70% of these tests failed. If invasive testing was not feasible, samples from the umbilical cord showed the highest success rates. Cytogenetic analysis was valuable in determining the cause of death in one fifth of intrauterine fetal deaths (18.7%).

Chromosomal abnormalities were established in 13% of intrauterine fetal deaths for which cytogenetic analysis was successful. This is higher than the 6-12% reported previously.[3,9,10,29] Furthermore, the 13% we found is in fact an underestimation, because investigation of missing inclusions indicated a selection bias for deaths

with known chromosomal abnormalities and congenital anomalies. However, the prevalence of the different chromosomal abnormalities we found versus those published earlier[30] was comparable. Parents with an intrauterine fetal death should be counseled about the prior probability of chromosomal abnormalities, which is at least 15-fold more than in livebirths.

Cytogenetic analysis was performed for 67.7% of the 750 intrauterine fetal deaths included in this study and was successful in 48.4%. The major reason this test was not performed in all the intrauterine fetal deaths was due to noncompliance with the study protocol. Others have reported overall successful chromosomal analysis in 41% of intrauterine fetal deaths[17] and 43% of stillborns.[9] Our highest success rates after death were for amniocentesis, CVS and invasive testing in combination with fetal, placental or umbilical cord biopsies. Amniocentesis and CVS were also the most successful in the study by Khare et al (90% and 100%, respectively)[19] and in other studies.[18,20] Identification of cytogenetic abnormalities during ongoing pregnancy compromised the completeness of the data in the post partum evaluation, although this has no consequences for our conclusions. A uniform approach to obtaining karyotypes in one center and culturing fibroblasts in a central laboratory could have raised our low culture success rate. Amniocenteses and CVS rates were not high in our study. It is not common practice in the Netherlands to perform these tests after intrauterine fetal death, probably due to ignorance, inexperience with the procedure, or missing logistics. A similar situation has also been reported for the United States.[2] Diminished or absent amniotic fluid (25%) may have also negatively influenced the decision to perform amniocentesis. In our experience, and in that of others, invasive testing after intrauterine fetal death is accepted well by parents.[19,29] In contrast to earlier published clinical guidelines,[11,13] we therefore recommend invasive cytogenetic analysis after intrauterine fetal death.

Alternatives to invasive cytogenetic analysis are fetal, placental or umbilical cord biopsies postpartum. However, these tissue cultures may fail to grow due to nonvital tissue or bacterial or fungal overgrowth, contamination during passage through the birth canal, inappropriate collection of biopsies, and transport problems. In our study, overall cytogenetic analysis of tissues solely postpartum was unsuccessful in 71.5% of cases. Success rates varied between 32.1% for umbilical cord and 0% for pericardium. Pericardium was, however, often represented in the "multiple" tissue group, which had a success rate of 25.4%. Success rates of skin and placenta biopsies (19.2%, combined in one group) were comparable to earlier published skin biopsy success rates of 18%[17] and 14%.[19] Much higher success rates in skin biopsies (76%) were established in pregnancy terminations for fetal abnormalities.[31] However, as success rates decrease with the duration of time that the fetus has been dead, skin biopsies for intrauterine

fetal death should be avoided in our opinion. Our success rates for cartilage were comparable to the 27.3% published for stillbirths from 16 weeks of gestation,[32] whereas placenta success rates (65.6%) in this study were much higher. Others have also advised placenta biopsies as they are often a successful source of viable fetal cells surviving for days after fetal death.[14,19,33,34] The few placenta biopsies included in our study might have led to a lower yield of successful cytogenetic analysis and our post partum failure rate might therefore be an overestimate. We only included a few placenta biopsies because earlier studies indicated that maternal cell contamination was a problem and placental mosaicism.[17] Other fetal tissue types in our cohort showed comparable success rates with other studies, although some reported even lower success rates of 7%.[17] We recommend cytogenetic analysis of umbilical cord postpartum as an alternative to invasive analysis, because this seems to have the highest culture success rate. Besides, sampling of umbilical cord is not as mutilating as sampling other tissue types. This is in contrast with earlier published advice that samples of fetal blood, skin, or fascia lata are good tissue sources.[2,10,11,13]

Additional molecular cytogenetic analyses, such as fluorescent in situ hybridization, multiplex ligation-dependent probe amplification, comparative genomic hybridization and quantitative fluorescent polymerase chain reaction, have been advised if cells do not grow in culture. Although the numbers in our study were small, these techniques seem promising; the majority of analyses were fluorescent in situ hybridization or multiplex ligation-dependent probe amplification since comparative genomic hybridization is more expensive. The feasibility of these techniques in common practice needs further evaluation.

Some authors recommend cytogenetic analysis for all fetal deaths,[9,10,29] while others have suggested that it be attempted in a selected group.[5,12,15,34,35] Prevalence of chromosomal abnormalities has been reported to be substantially higher in the presence of congenital anomalies.[5,35-38] Some morphologic abnormalities will have been diagnosed at ultrasonography. However, interpretation of ultrasonography after intrauterine fetal death is not always conclusive. Prevalence of morphologic abnormalities determined at birth (27.8%) in our cohort was higher than the 20% reported earlier.[9,39] Determination of these abnormalities is indeed significant for predicting abnormal chromosomes. However, whereas most chromosomally aberrant fetuses show some morphologic abnormalities, in our study, 24% did not, and previously a figure of 10% was reported.[9] In morphologically normal fetuses, the posterior probability of a chromosomal abnormality in our study was 4.6%, one-third of our prior probability (13%). Others expected this to be <2%.[10] In the presence of morphologic abnormalities, the posterior probability of a chromosomal abnormality was 38%. Others reported 25-50% fetal aneuploidy in the context of fetal abnormalities noted at postmortem examination.[10,40]

Our perinatal clinical geneticist observed some common variants diagnosed as morphologic abnormalities by the physician, illustrating an interobserver variation. A new prediction of the missed chromosomal abnormalities in morphologically normal fetuses reduced the missed chromosomal abnormalities to 4.4%. First-hand evaluation of morphologic abnormalities by trained pediatricians, geneticists or pathologists would probably have given better posterior probability results, but this was not feasible in our study. However, if morphologic abnormalities are determined after birth there only remains the option of postpartum cytogenetic analysis and much effort is required for little result. Parents who object to invasive cytogenetic analysis need to be counseled about these consequences, whereas after birth, the presence of morphologic abnormalities may influence the parents' decision to allow cytogenetic analysis. Some may not find the level of 4.6% chromosomal abnormalities seen in morphologically normal fetuses acceptable.

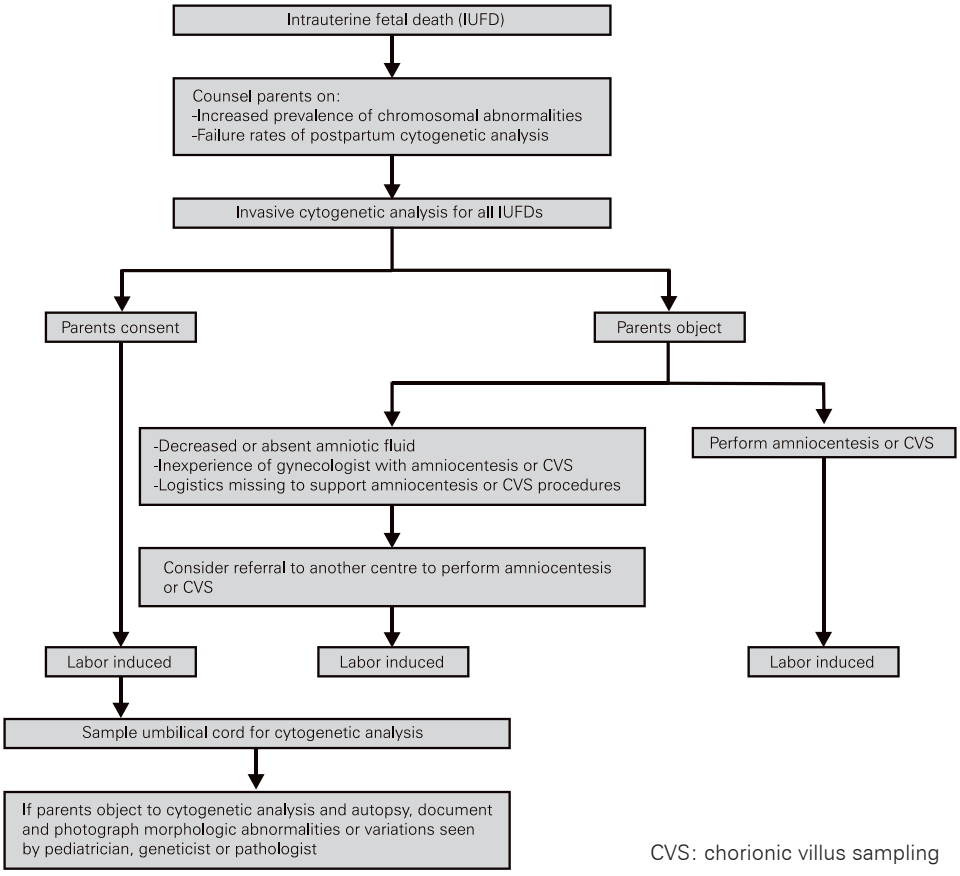
In our study the chances of fetal aneuploidy were not increased for SGA fetuses, advanced maternal age (older than 35 years) or decreased gestational age, in contrast to results reported by others.[10,14,19] Neither did we find SGA or advanced maternal age to be an adequate selection criterion for performing cytogenetic analysis. However, because we excluded intrauterine fetal deaths at 20-24 weeks from our SGA analysis, we could have introduced some bias. Khare et al found most chromosomal abnormalities in the second trimester. If resources are limited, they advised cytogenetic analysis for this group and for the third trimester if the intrauterine fetal death is associated with fetal anomalies.[19] We disagree here, as we found more than one half of our chromosomal abnormalities in third-trimester intrauterine fetal deaths.

Some argue that cytogenetic analysis after intrauterine fetal death should only be attempted in non-macerated or mildly macerated fetuses,[41,42] again, we disagree. Similar to other reports, we found no significant differences in success rates between mildly, moderately or severely macerated fetuses.[9,33] Furthermore, we would have missed 20 chromosomal abnormalities if no effort had been made to assess macerated fetuses using cytogenetic analysis.

The decision to perform any test should be based on the value of the test, its cost and the resources involved, the risk of harm by the test, the ease with which the test can be performed, the impact of missing a positive result, and the diagnostic discrimination of the particular test.[29] On most points, cytogenetic analysis for all intrauterine fetal deaths seems justified. Cost aspects were not evaluated in our study but others have done this and concluded that if nonselective stillbirth assessment is performed, new information relevant to a recurrence risk estimation, prenatal diagnosis recommendation, or preconceptual and prenatal treatment will be gained from 51% of stillborns.[43]

On the basis of our results we have drawn up a flowchart (Figure 1) illustrating the clinical implications of our findings. We recommend invasive cytogenetic analysis as standard diagnostic workup after intrauterine fetal death. The flowchart also addresses the issues which should be presented to parents during counseling and some alternative options with their consequences. The risk of a chromosomal abnormality in an intrauterine fetal death cohort is substantially higher than for most cases of prenatal testing. Cytogenetic analysis after intrauterine fetal death, regardless of whether morphologic abnormalities are present, should be funded. Waiting to take samples for cytogenetic testing until after the birth means results are missed unnecessarily and money is wasted. Gaining skills and experience in invasive cytogenetic analysis should be part of the training program for all gynecologists.

Figure 1. Flowchart for cytogenetic analysis after intrauterine fetal death



CVS: chorionic villus sampling

REFERENCES

1. Alessandri LM, Stanley FJ, Newnham J, Walters BN. The epidemiological characteristics of unexplained antepartum stillbirths. Early Hum Dev. 1992;30:147-161.
2. Fretts RC. Etiology and prevention of stillbirth. Am J Obstet Gynecol. 2005;193:1923-1935.
3. Goldenberg RL, Kirby R, Culhane JF. Stillbirth: a review. J Matern Fetal Neonatal Med. 2004;16:79-94.
4. Maternal and Child Health Consortium. CESDI 8th annual report: Confidential Enquiry of Stillbirths and Deaths during Infancy. 2001. London.
5. Pitkin RM. Fetal death: diagnosis and management. Am J Obstet Gynecol. 1987;157:583-589.
6. Buitendijk S, Zeitlin J, Cuttini M, Langhoff-Roos J, Bottu J. Indicators of fetal and infant health outcomes. Eur J Obstet Gynecol Reprod Biol. 2003;111 Suppl 1:S66-S77.
7. Gardosi J, Kady SM, McGeown P, Francis A, Tonks A. Classification of stillbirth by relevant condition at death (ReCoDe): population based cohort study. BMJ. 2005;331:1113-1117.
8. Kramer MS, Liu S, Luo Z, Yuan H, Platt RW, Joseph KS. Analysis of perinatal mortality and its components: time for a change? Am J Epidemiol. 2002;156:493-497.
9. Pauli RM, Reiser CA, Lebovitz RM, Kirkpatrick SJ. Wisconsin Stillbirth Service Program: I. Establishment and assessment of a community-based program for etiologic investigation of intrauterine deaths. Am J Med Genet. 1994;50:116-134.
10. Silver RM. Fetal death. Obstet Gynecol. 2007;109:153-167.
11. ACOG Committee Opinion No. 383: Evaluation of stillbirths and neonatal deaths. Obstet Gynecol. 2007;110:963-966.
12. Curry CJ, Honore LH. A protocol for the investigation of pregnancy loss. Clin Perinatol. 1990;17:723-742.
13. Leduc L, Farine D, Armson BA et al. Stillbirth and bereavement: guidelines for stillbirth investigation. J Obstet Gynaecol Can. 2006;28:540-552.
14. Menasha J, Levy B, Hirschhorn K, Kardon NB. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. Genet Med. 2005;7:251-263.
15. Mueller RF, Sybert VP, Johnson J, Brown ZA, Chen WJ. Evaluation of a protocol for post-mortem examination of stillbirths. N Engl J Med. 1983;309:586-590.
16. European Cytogeneticists Association. E.C.A News Letter. 17, 1-32. 2006. Hannover.
17. 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.
18. Brady K, Duff P, Harlass FE, Reid S. Role of amniotic fluid cytogenetic analysis in the evaluation of recent fetal death. Am J Perinatol. 1991;8:68-70.
19. Khare M, Howarth E, Sadler J, Healey K, Konje JC. A comparison of prenatal versus postnatal karyotyping for the investigation of intrauterine fetal death after the first trimester of pregnancy. Prenat Diagn. 2005;25:1192-1195.
20. Neiger R, Croom CS. Cytogenetic study of amniotic fluid in the evaluation of fetal death. J Perinatol. 1990;10:32-34.

21. RCOG. Fetal and perinatal pathology. Report of a joint working party. RCOG. 2001. London.

22. Bove KE. Practice guidelines for autopsy pathology: the perinatal and pediatric autopsy. Autopsy Committee of the College of American Pathologists. Arch Pathol Lab Med. 1997;121:368-376.

23. Langston C, Kaplan C, Macpherson T et al. Practice guideline for examination of the placenta: developed by the Placental Pathology Practice Guideline Development Task Force of the College of American Pathologists. Arch Pathol Lab Med. 1997;121:449-476.

24. Merks JH, van Karnebeek CD, Caron HN, Hennekam RC. Phenotypic abnormalities: terminology and classification. Am J Med Genet A. 2003;123:211-230.

25. Merks JH, Ozgen HM, Cluitmans TL et al. Normal values for morphological abnormalities in school children. Am J Med Genet A. 2006;140:2091-2109.

26. Kloosterman GJ. On intrauterine growth. The significance of prenatal care. Int J Gynaecol Obstet. 1970;895-912.

27. Wigglesworth JS, Singer DB. Textbook of fetal and perinatal pathology. Second ed. Blackwell Science; 1998.

28. Korteweg FJ, Gordijn SJ, Timmer A et al. The Tulip classification of perinatal mortality: introduction and multidisciplinary inter-rater agreement. BJOG. 2006;113:393-401.

29. Petersson K, Bremme K, Bottinga R et al. Diagnostic evaluation of intrauterine fetal deaths in Stockholm 1998-99. Acta Obstet Gynecol Scand. 2002;81:284-292.

30. Wapner RJ, Lewis D. Genetics and metabolic causes of stillbirth. Semin Perinatol. 2002;26:70-74.

31. Kyle PM, Sepulveda W, Blunt S, Davies G, Cox PM, Fisk NM. High failure rate of postmortem karyotyping after termination for fetal abnormality. Obstet Gynecol. 1996;88:859-862.

32. Baena N, Guitart M, Ferreres JC et al. Fetal and placenta chromosome constitution in 237 pregnancy losses. Ann Genet. 2001;44:83-88.

33. Doyle EM, McParland P, Carroll S, Kelehan P, Mooney EE. The role of placental cytogenetic cultures in intrauterine and neonatal deaths. J Obstet Gynaecol. 2004;24:878-880.

34. Incerpi MH, Miller DA, Samadi R, Settlage RH, Goodwin TM. Stillbirth evaluation: what tests are needed? Am J Obstet Gynecol. 1998;178:1121-1125.

35. Ellis PM, Bain AD. Cytogenetics in the evaluation of perinatal death. Lancet. 1984;1:630-631.

36. Angell RR, Sandison A, Bain AD. Chromosome variation in perinatal mortality: a survey of 500 cases. J Med Genet. 1984;21:39-44.

37. Sutherland GR, Carter RF. Cytogenetic studies: an essential part of the paediatric necropsy. J Clin Pathol. 1983;36:140-142.

38. Yusuf RZ, Naeem R. Cytogenetic abnormalities in products of conception: a relationship revisited. Am J Reprod Immunol. 2004;52:88-96.

39. Pauli RM, Reiser CA. Wisconsin Stillbirth Service Program: II. Analysis of diagnoses and diagnostic categories in the first 1,000 referrals. Am J Med Genet. 1994;50:135-153.

40. Winter RM, Knowles SAS, Bieber FR, Baraitser M. The malformed fetus and stillbirth: a diagnostic approach. In: Chromosome abnormalities in stillbirths, perinatal deaths and spontaneous abortions. Chichester and Sons; 1988.

41. Macpherson TA, Garver KL, Turner JH, Diggans GR, Marchese SG, Poole GC. Predicting in vitro tissue culture growth for cytogenetic evaluation of stillborn fetuses. Eur J Obstet Gynecol Reprod Biol. 1985;19:167-174.

42. Smith A, Bannatyne P, Russell P, Ellwood D, den DG. Cytogenetic studies in perinatal death. Aust N Z J Obstet Gynaecol. 1990;30:206-210.

43. Michalski ST, Porter J, Pauli RM. Costs and consequences of comprehensive stillbirth assessment. Am J Obstet Gynecol. 2002;186:1027-1034.

Chapter 7

Fetal loss in women with hereditary deficiencies of antithrombin, protein C or protein S, and the contribution of cosegregation of other thrombophilic defects

Submitted

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ABSTRACT

Background

Hereditary deficiencies of antithrombin (AT), protein C (PC) and protein S (PS) are strong risk factors for venous thromboembolism (VTE). The risk is reinforced by cosegregation of other thrombophilic defects. Single deficiencies and cosegregation may similarly increase the risk of fetal loss due to placental thrombosis.

Design and Methods

In a retrospective family cohort study, we assessed the absolute risk of fetal loss, comparing deficient women to non-deficient relatives.

Results

Of 630 women, 317 were evaluable, who had 987 pregnancies (582 in 185 deficient women). Total fetal loss rates were 47% (AT deficient), 45% (PC deficient), 21% (PS type I deficient) and 30% (PS type III deficient), compared to 32%, 28%, 29% and 27% in non-deficient women, respectively. Adjusted relative risks were 2.3 (95% CI, 0.9-6.1), 2.1 (0.9-4.7), 0.7 (0.2-1.8) and 1.1 (0.6-2.0). Differences were mainly due to higher late fetal loss rates in AT deficient (adjusted relative risk 11.3; 95% CI, 3.0-42.0) and PC deficient women (4.7; 1.3-17.4). Cosegregation of factor V Leiden and prothrombin G20210A did not increase the risk, neither in deficient women (29% vs. 34%), nor in non-deficient women (24% vs. 28%). This was explained by excluding pregnancies after prior VTE. In excluded women, total fetal loss rates were 39% in deficient women and 0% in non-deficient women.

Conclusions

Hereditary AT and PC deficiencies were associated with a high absolute risk of fetal loss. An additional effect of cosegregation was not demonstrated, may be due to the exclusion of women at highest risk of VTE.

INTRODUCTION

Hereditary deficiencies of antithrombin (AT), protein C (PC) and protein S (PS) are strong risk factors for venous thromboembolism (VTE).[1-3] Women with these deficiencies are at higher risk of VTE during pregnancy and puerperium, due to the acquired hypercoagulable state associated with this condition.[4-6] It is likely that not only maternal veins but also placental vessels are more prone to the development of thrombosis, as has been demonstrated in women with mild thrombophilic defects.[7-10] Consequently, women with strong thrombophilic defects, i.e. deficiencies of AT, PC or PS, may be at higher risk of fetal loss, due to placental insufficiency as a result of placental infarction. Thus far, only a few family studies addressed fetal loss in women with these rare deficiencies.[11-14] Although the reported risk of fetal loss was increased compared to controls,[11,12] this result was not consistent in meta-analyses.[13,14]

Recently, it was demonstrated that concomitance of other thrombophilic defects increases the risk of VTE in subjects with hereditary deficiencies of AT, PC or PS. Moreover, concomitance was frequently observed in families with these deficiencies.[3,15-17] Similarly, concomitance might also increase the risk of fetal loss. We performed a study to assess the absolute risk of fetal loss associated with hereditary deficiencies of AT, PC and PS, and the contribution of additional thrombophilic defects to this risk.

DESIGN AND METHODS

We studied women from a retrospective study of families with hereditary deficiencies of either AT, PC, PS type I, or PS type III.[2,3] Probands in that study were consecutive patients with documented VTE in whom one of these deficiencies was demonstrated. They were referred with clinically suspected VTE to our thrombosis out-patient clinic over a period of 12 years. First-degree relatives older than 15 years of age were identified. As the number of AT deficient probands was small, second degree relatives from a deficient parent were also identified. The study was approved by the institutional review board of our hospital. Informed consent was obtained from all participants. In the present study, women were eligible if they had been pregnant at least once resulting in live birth or fetal loss, excluding ectopic and terminated pregnancies. Detailed information about episodes of venous thromboembolism and course and outcome of previous pregnancies from 15 years of age up to enrolment was collected using a standardized questionnaire and by reviewing medical records. We defined early fetal loss as loss up to 22 weeks of gestation and late fetal loss as loss after 22 completed weeks of gestation,

according to the criteria of the World Health Organisation.[18] Recurrent fetal loss was defined as two or more losses. Blood samples for testing on thrombophilic defects were taken after clinical data had been collected. These tests included factor V Leiden and the prothrombin G20210A mutation, in addition to all above mentioned deficiencies.

Laboratory studies

AT activity (Coatest, Chromogenix, Mölndal, Sweden) and PC activity (Berichrom Protein C; Dade Behring, Marburg, Germany) were measured by chromogenic substrate assays, PC antigen levels and total and free PS antigen were measured by Enzyme Linked Immuno Sorbent Assay (DAKO, Glostrup, Denmark). Normal ranges were determined in 393 healthy blood donors, who had no (family) history of venous or arterial thromboembolism and were neither pregnant, nor had used oral contraceptives for at least three months. AT deficiency was defined by levels below the lower limit of its normal range (<74 IU/dl), PC deficiency type I and type II by lowered levels of either PC antigen (<63 IU/dl) and/or activity (<64 IU/dl). PS deficiency type I was defined by total (<67 IU/dl) and free PS levels (< 65 IU/dl) below the lower limit of their normal ranges, and type III deficiency by lowered free PS levels and normal total PS levels. Deficiencies were considered inherited if confirmed at repeated measurements of samples collected at a three month interval and demonstrated in at least two family members, while acquired conditions were excluded. A deficiency was considered acquired and due to oral contraception or pregnancy, unless it was confirmed at least three months after discontinuation of oral contraception and delivery, respectively. Factor V Leiden and the prothrombin G20210A mutation were demonstrated by polymerase chain reactions.[19,20]

In probands and relatives, who had had VTE, blood samples were collected at least three months after this event had occurred. If they were still treated with a vitamin K antagonist, samples were taken after this therapy had been interrupted, meanwhile nadroparin was given subcutaneously.

Statistical analysis

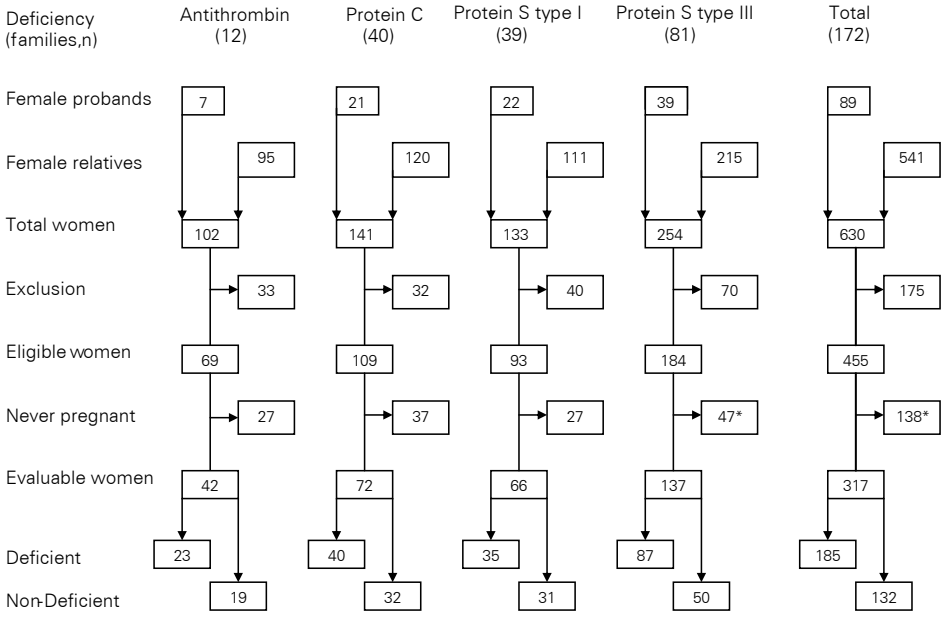
Absolute fetal loss risks were expressed as percentages of pregnancies ending in fetal loss and percentages of women with fetal loss prior to enrolment i.e. prior to their classification as deficient or non-deficient. Probands and deficient relatives were compared to non-deficient relatives. Relative risks were adjusted for clustering of pregnancies in women by random effects logistic regression. Considering the high risk of VTE in women with hereditary deficiencies and the probability that they had received thromboprophylaxis during pregnancies after

prior VTE, we excluded pregnancies after a prior episode of VTE from analysis, because thromboprophylaxis might have influenced outcome of these pregnancies. As a consequence, women without a pregnancy before first VTE were excluded. The effect of concomitant thrombophilic defects was assessed for factor V Leiden and the prothrombin G20210A mutation, comparing deficient and non-deficient women. Continuous variables were expressed as median values and ranges and categorical data as counts and percentages. Differences between groups for continuous data were evaluated by using the Student t test or Mann-Whitney U test, depending on the normality of data and by using the Fisher exact test or Chi Square test for categorical data. A two-tailed p-value < 0.05 was considered to indicate statistical significance. Statistical analyses were performed using SAS software, version 9.1 (SAS-Institute Inc., Cary, NC, USA).

RESULTS

Overall, of 89 female probands and 541 female relatives, 175 women were excluded due to age < 15 years, death, geographic distance and refused consent. Eligible were 455 women of whom 136 were never pregnant and 2 women had only ectopic

Figure 1. Recruitment of women from families with hereditary deficiencies of either antithrombin, protein C or protein S.



* two women only had ectopic or terminated pregnancies

or terminated pregnancies. The remaining 317 women were analyzed of whom 185 were deficient and 132 were non-deficient (Figure 1). Their clinical characteristics are summarized in Table 1. Overall, median age at first pregnancy in deficient and non-deficient women was comparable. Fetal loss rates in deficient women and non-deficient women were 36% and 28%, respectively (p=0.18). These were comparable in probands and deficient relatives (p=0.64). Deficient women of each cohort showed higher fetal loss rates then their non-deficient relatives, particularly in the cohorts of AT deficient and PC deficient families. Of deficient women (including probands), 43% had VTE at fertile age, compared to 9% of non-deficient women (p<0.001). This difference was less pronounced in the cohort of PS type III deficient families (26% vs. 14%; p=0.13). Cosegregation of factor V Leiden and/or the prothrombin G20210A mutation was demonstrated in 24% of deficient women and in 18% of non-deficient women (p=0.40). Figure 2 shows the distribution of fetal loss related to gestational age in deficient and non-deficient women for the separate cohorts of AT-, PC-, PS type I-, and PS type III-deficient families.

Table 2 shows absolute risks of fetal loss, excluding pregnancies after prior VTE. Total fetal loss rates were 47% in AT deficient women, 45% in PC deficient women, 21% in PS type I deficient women, and 30% in PS type III deficient women, compared to 32%, 28%, 29% and 27% in non-deficient women, respectively. Adjusted for clustering of pregnancies in women, and compared to all non-deficient women, relative risks were 2.3 (95% CI, 0.9-6.1) in AT deficient women, 2.1 (95% CI, 0.9-4.7) in PC deficient women, 0.7 (95% CI, 0.2-1.8) in PS type I deficient women, and 1.1 (95% CI, 0.6-2.0) in PS type III deficient women. Early fetal loss rates showed no statistically significant differences between deficient and non-deficient women. Late fetal loss rates were 32% and 16% in AT deficient and PC deficient women, respectively; adjusted relative risks were 11.3 (95% CI, 3.0-42.0) and 4.7 (95% CI, 1.3-17.4). In PS type I and PS type III deficient women, late fetal loss rates were 3% and 7%, respectively; adjusted relative risks were 0.9 (95% CI, 0.1-7.8) and 1.9 (95% CI, 0.6-6.4).

Of 289 women, who were included in our analysis, 273 (94%) were tested on factor V Leiden and the prothrombin G20210A mutation. Cosegregation was demonstrated in 52 women (19%). Of women with cosegregation, 6% were double heterozygotes. Total fetal loss rates were 29% in deficient women with cosegregation and 34% in deficient women without cosegregation. In non-deficient women these were 24% and 28%, respectively. Differences were not statistically significant. Cosegregation was demonstrated in 43% of 28 excluded women. Of women with cosegregation 50% were double heterozygotes. Total fetal loss rates in excluded women were 39% in deficient women and 0 % in non-deficient women.

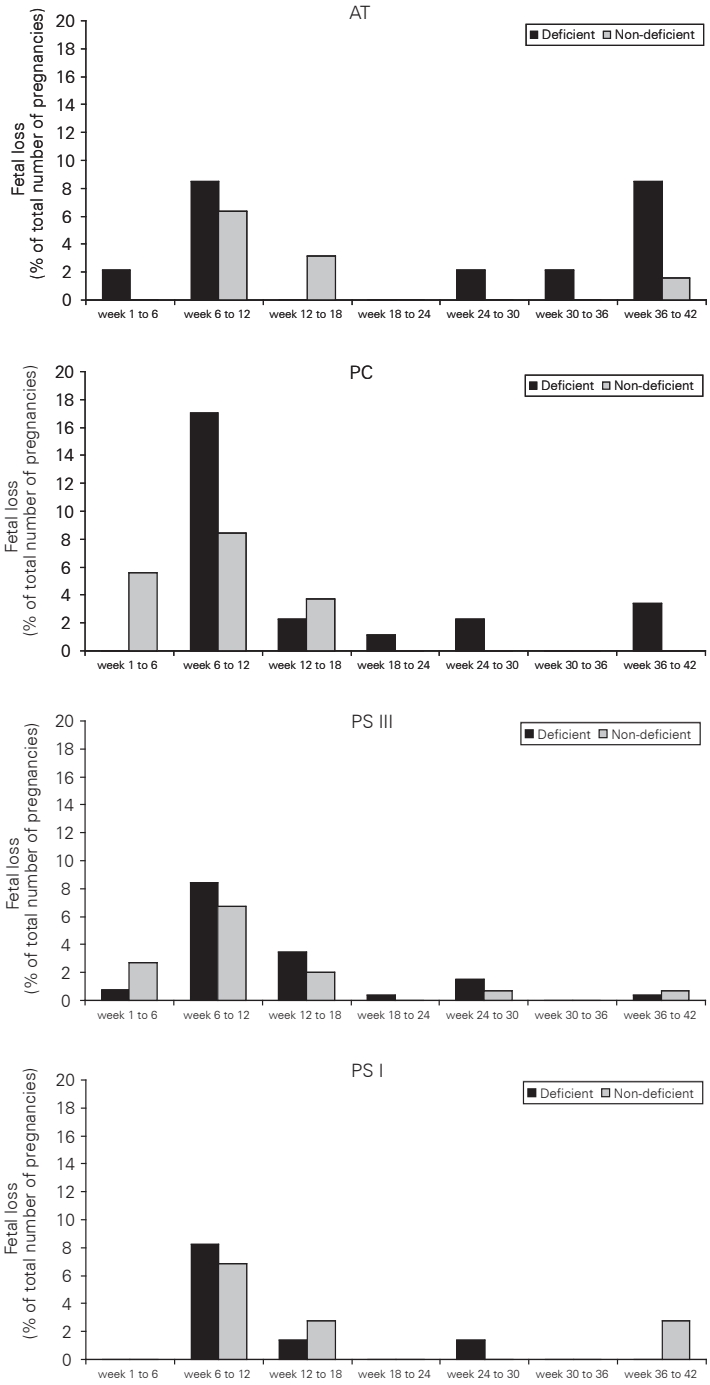


Figure 2. Distribution of fetal loss related to gestational age in deficient and non-deficient women for the separate cohorts of antithrombin (AT), protein C (PC), protein S type I (PSI) , and protein S type II (PSIII) deficient families.

Table 1. Clinical characteristics of 317 women with hereditary deficiencies of either antithrombin, protein C or protein S, and their non-deficient female relatives.

	Antithrombin		Protein C	
	Def.	**Non-def.**	**Def.**	**Non-def.**
Women, n	23	19	40	32
Age at first pregnancy, median (range)	27 (16-34)	25 (18-40)	24 (15-33)	25 (17-32)
Fetal loss, n (%)	9 (39)	6 (32)	20 (50)	9 (28)
Recurrent fetal loss, n (%)	2 (9)	1 (5)	6 (15)	6 (19)
VTE at fertile age, n (%)	13 (57)	0	22 (55)	1 (3)
Cosegregation, n/n (%)*	3/20 (15)	3/15 (20)	10/38 (26)	5/29 (17)
Pregnancies, n	68	63	123	107
Pregnancies/woman, median (range)	2 (1-7)	3 (1-10)	3 (1-8)	3 (1-11)
Fetal loss, n (%)	12 (18)	7 (11)	33 (27)	19 (18)

Def. denotes deficient. VTE, venous thromboembolism *Cosegregation of factor V Leiden and/or prothrombin G20210A; n affected/n tested (%)

Table 2. Total, early and late fetal loss rates in 289 women with hereditary deficiencies of either antithrombin, protein C or protein S and their non-deficient relatives, excluding pregnancies after prior VTE in 28 women.

	Pregnancies	
	n	**Fetal loss**
Antithrombin, n (%)		
Deficient	47	11 (23)
Non-deficient	63	7 (11)
Adjusted RR* (95% CI); p		
Protein C, n (%)		
Deficient	88	23 (26)
Non-deficient	107	19 (18)
Adjusted RR* (95% CI); p		
Protein S type I, n (%)		
Deficient	73	8 (11)
Non-deficient	73	9 (12)
Adjusted RR* (95% CI); p		
Protein S type III, n (%)		
Deficient	261	39 (15)
Non-deficient	148	19 (13)
Adjusted RR* (95% CI); p		

*Relative risk (RR) adjusted for clustering of pregnancies in women, and compared to all non-deficient women. CI, confidence interval

Protein S type I		Protein S type III		Total	
Def	**Non-def.**	**Def.**	**Non-def.**	**Def.**	**Non-def.**
35	31	87	50	185	132
26 (17-34)	26 (18-32)	24 (17-37)	26 (16-37)	25 (15-37)	25 (16-40)
10 (29)	8 (26)	27 (31)	14 (28)	66 (36)	37 (28)
3 (9)	1 (3)	11 (13)	3 (6)	22 (12)	11 (8)
21 (60)	4 (13)	23 (26)	7 (14)	79 (43)	12 (9)
9/33 (27)	4/30 (13)	20/87 (23)	10/49 (20)	42/178 (24)	22/123 (18)
107	82	284	155	582	407
3 (1-9)	3 (1-6)	3 (1-9)	3 (1-9)	3 (1-9)	3 (1-11)
15 (14)	9 (11)	43 (15)	20 (13)	103 (18)	55 (14)

		Women		
Excluded	**Analysed**	**Total fetal loss**	**Early fetal loss**	**Late fetal loss**
4 (17)	19	9 (47)	4 (21)	6 (32)
0 (0)	19	6 (32)	5 (26)	1 (5)
		2.3 (0.9-6.1); 0.10	0.8 (0.2-2.6); 0.70	11.3 (3.0-42.0); 0.0003
9 (23)	31	14 (45)	11 (35)	5 (16)
0 (0)	32	9 (28)	9 (28)	0
		2.1 (0.9-4.7); 0.07	1.6 (0.7-3.8); 0.25	4.7 (1.3-17.4); 0.02
6 (17)	29	6 (21)	5 (17)	1 (3)
3 (10)	28	8 (29)	6 (21)	2 (7)
		0.7 (0.2-1.8); 0.40	0.6 (0.2-1.8); 0.37	0.9 (0.1-7.8); 0.90
4 (5)	83	25 (30)	22 (27)	6 (7)
2 (4)	48	13 (27)	12 (25)	2 (4)
		1.1 (0.6-2.0); 0.78	1.1 (0.6-2.0); 0.83	1.9 (0.6-6.4); 0.30

DISCUSSION

This study showed a high absolute risk of fetal loss in women with hereditary deficiencies of AT or PC. In women with hereditary deficiencies of PS type I or type III, the risk was comparable to non-deficient women. Cosegregation of factor V Leiden and/or the prothrombin G20210A mutation apparently did not influence the risk of fetal loss.

The absolute risk of total fetal loss in our study was 47% in AT deficient women and 45% in PC deficient women, being 2.3 and 2.1-fold higher than in non-deficient women. Previous studies showed odds ratios of total fetal loss ranging from 1.5 to 2.5 in AT deficient women and from 1.4 to 2.5 in PC deficient women, compared to controls.[11-13] Although relative risks in our study were in agreement with previous studies, absolute risks in AT non-deficient and PC non-deficient relatives (32% and 28%, respectively) were higher than in controls (24%) reported by Preston et al. Controls in the latter study were partners of male participants of the EPCOT cohort or acquaintances of cases. As we compared deficient women to their non-deficient relatives, cosegregation of other thrombophilic defects in these families might explain the higher risk of fetal loss in non-deficient, as well as deficient women. The higher risk of total fetal loss in AT and PC deficient women in our study was mainly due to an 11.3 and 4.7-fold increased late fetal loss rate, while early fetal loss was comparable to non-deficient relatives. Preston et al, showed an odds ratio for early fetal loss of 1.7 (95% CI, 1.0-2.8) in AT deficient women and 1.4 (95% CI, 0.9-2.2) in PC deficient women, while this was 5.2 (95% CI, 1.5-18.1) for late fetal loss in AT deficient women and 2.3 (95% CI, 0.6-8.3) in PC deficient women.[11]

We observed the lowest risk for total fetal loss in PS type I deficient and PS type III deficient women. Total fetal loss risks, as well as early and late fetal loss risks were comparable to non-deficient relatives for both types of PS deficiency. Preston et al. found a comparable odds ratio of 1.3 (95% CI 0.8-2.1) for total fetal loss in PS deficient women, while it was 3.3 (95% CI, 1.0-11.3) for late fetal loss.[11] In a meta-analysis the risk for total fetal loss was even 7.4-fold higher (95% CI, 1.3-42.8).[13] In contrast with previous studies, we separately assessed the risk of fetal loss in women with type I and type III PS deficiency, because we previously had demonstrated that type III PS deficiency was not a risk factor for VTE.[2] The assumption that it might also not be associated with an increased risk for fetal loss was supported by our data. It is remarkable, however that PS deficiency type I did not influence the risk of fetal loss, considering that it is comparable to AT deficiency and PC deficiency as a risk factor for VTE.[5-6]

In our study, cosegregation of factor V Leiden and the prothrombin G20210A mutation apparently did not influence the risk of fetal loss neither in deficient nor in non-deficient women. In fact, we observed a lower rather than higher risk of

fetal loss in deficient women with cosegregation, though numbers were small. Exclusion of pregnancies after prior VTE could be an explanation for this finding. Cosegregation of other thrombophilic defects results in a higher risk and earlier onset of VTE in subjects with deficiencies of AT, PC or PS.[3] One would expect that cosegregation also had increased the risk of fetal loss.[11] By excluding pregnancies after prior VTE from analysis, we probably excluded women at highest risk of VTE, i.e. deficient women with cosegregation, and consequently women with potentially the highest risk of fetal loss. Indeed, cosegregation was more frequently observed in excluded women than in analyzed women, whereas the former showed a higher total fetal loss rate. Our assumption that thromboprophylaxis during pregnancy in deficient women reduced the estimated risk of fetal loss was further supported by the previously reported results of a prospective observational study on the same family cohort.[21] That study showed a fetal loss rate of 0% in deficient women, who received thromboprophylaxis during pregnancy, compared to 45% in deficient women who did not ($p=0.001$).

A comparison of our results with previous reports on fetal loss related to thrombophilic deficiencies and other thrombophilic defects is hampered by differences in methodology. The majority of previous studies addressed the incidence of thrombophilic defects in women with adverse pregnancy outcomes.[7,9,13,14,22-24] We assessed fetal loss rates in families with hereditary deficiencies, which were identified by testing consecutive patients with VTE. Furthermore, gestational ages ranged widely in previous studies and some studies did not differentiate between early and late fetal loss. However, placental function depends on gestational age,[25,26] and mechanisms of early and late fetal loss are different. Although placental thrombosis is a plausible explanation for (late) fetal loss in deficient women, deficiencies of AT, PC and PS may also contribute to another pathophysiological mechanism. Experiments in mice provided evidence that fibrin degradation products induce apoptosis of trophoblasts, resulting in fetal loss.[27] As it is likely that deficiencies of AT, PC and PS are associated with increased generation of thrombin and, consequently, fibrin and fibrin degradation products, we speculate that deficient women will be more prone to fetal loss than non-deficient women. In deficient women, early fetal loss may be due to apoptosis of trophoblasts, while late fetal loss may be a result of placental thrombosis. In accordance with this hypothesis, anticoagulant treatment during pregnancy might have a beneficial effect on both early and late fetal loss in deficient women, as suggested by the results of a prospective study mentioned before.[21]

This study has obvious limitations. The absolute risk of fetal loss in deficient women may have been underestimated by excluding pregnancies after prior VTE and consequently women at higher risk of fetal loss. Although a systematic search for other causes of early and late fetal loss was not performed due to the retrospective

design of our study, it is likely that these were equally distributed among deficient and non-deficient women. Recall bias regarding fetal loss may have been introduced by its retrospective design, but its influence remained limited as clinical data was collected prior to classification of women as deficient or non-deficient. In addition, patients were not selected because of their compromised obstetrical history. Referral bias cannot be excluded by the setting of a university hospital. Important is how patients were selected, they all had a family or personal history of VTE and were therefore at risk of fetal loss. Although probands were included, selection bias is not very likely, as consecutive patients with VTE were tested to identify probands and their relatives rather than women with fetal loss.

In conclusion, hereditary deficiencies of AT and PC were associated with an excessively high absolute risk of (late) fetal loss, in contrast with PS deficiency. An additional effect of cosegregation of other thrombophilic defects, though plausible, was not demonstrated, maybe due to excluding women at the highest risk of VTE.

Acknowledgments

We dedicate this manuscript to Jan van der Meer, last author of this manuscript who died unexpectedly on 14th January 2009.

REFERENCES

1. Sanson BJ, Simioni P, Tormene D et al. The incidence of venous thromboembolism in asymptomatic carriers of a deficiency of antithrombin, protein C, or protein S: a prospective cohort study. Blood. 1999;94:3702-6.
2. Brouwer JL, Veeger NJ, van der Schaaf W, Kluin-Nelemans HC, Meer J. Difference in absolute risk of venous and arterial thrombosis between familial protein S deficiency type I and type III. Results from a family cohort study to assess the clinical impact of a laboratory test-based classification. Br J Haematol. 2005;128:703-10.
3. Brouwer JL, Veeger NJ, Kluin-Nelemans HC, Meer J. The pathogenesis of venous thromboembolism: evidence for multiple interrelated causes. Ann Intern Med. 2006;145:807-15.
4. Conard J, Horellou MH, Van Dreden P, Lecompte T, Samama M. Thrombosis and pregnancy in congenital deficiencies in AT III, protein C or protein S: study of 78 women. Thromb Haemost. 1990;63:319-20.
5. De Stefano V, Leone G, Mastrangelo S et al. Thrombosis during pregnancy and surgery in patients with congenital deficiency of antithrombin III, protein C, protein S. Thromb Haemost. 1994;71:799-800.
6. Folkeringa N, Brouwer JL, Korteweg FJ et al. High risk of pregnancy-related venous thromboembolism in women with multiple thrombophilic defects. Br J Haematol. 2007;138:110-6.
7. Rai RS, Regan L, Chitolie A, Donald JG, Cohen H. Placental thrombosis and second trimester miscarriage in association with activated protein C resistance. Br J Obstet Gynaecol. 1996;103:842-4.
8. Kupferminc MJ, Eldor A, Steinman N et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med. 1999;340:9-13.
9. Meinardi JR, Middeldorp S, de Kam PJ et al. Increased risk for fetal loss in carriers of the factor V Leiden mutation. Ann Intern Med. 1999;130:736-9.
10. Martinelli I, Taioli E, Cetin I et al. Mutations in coagulation factors in women with unexplained late fetal loss. N Engl J Med. 2000;343:1015-8.
11. Preston FE, Rosendaal FR, Walker ID et al. Increased fetal loss in women with heritable thrombophilia. Lancet. 1996;348:913-6.
12. Sanson BJ, Friederich PW, Simioni P et al. The risk of abortion and stillbirth in antithrombin-, protein C-, and protein S-deficient women. Thromb Haemost. 1996;75:387-8.
13. Rey E, Kahn SR, David M, Schrier I. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet. 2003;361:901-8.
14. Robertson L, Wu O, Langhorne P et al. The Thrombosis Risk and Economic Assessment of Thrombophilia Screening (TREATS) Study. Thrombophilia in pregnancy: a systematic review. Br J Haematol. 2006;132:171-96.
15. Koeleman BP, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families. Blood. 1994;84:1031-5.
16. van Boven HH, Reitsma PH, Rosendaal FR et al. Factor V Leiden (FV R506Q) in families with inherited antithrombin deficiency. Thromb Haemost. 1996;75:417-21.

17. Zöller B, Berntsdotter A, García de Frutos P, Dahlback B. Resistance to activated protein C as an additional genetic risk factor in hereditary deficiency of protein S. Blood. 1995;85:3518-23.

18. Stirrat G.M. Recurrent miscarriage. Lancet. 1990;336:673-5.

19. Bertina RM, Koeleman BP, Koster T et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994;369:64-7.

20. Danneberg J, Abbes AP, Bruggeman BJ et al. Reliable genotyping of the G-20210-A mutation of coagulation factor II (prothrombin). Clin Chem. 1998;44:349-51.

21. Folkeringa N, Brouwer JL, Korteweg FJ et al. Reduction of high fetal loss rate by anticoagulant treatment during pregnancy in antithrombin, protein C or protein S deficient women. Br J Haematol. 2007;136:656-61.

22. Pabinger I, Vormittag R. Thrombophilia and pregnancy outcomes. J Thromb Haemost. 2005;3:1603-10.

23. Alonso A, Soto I, Urgellés MF et al. Acquired and inherited thrombophilia in women with unexplained fetal losses. Am J Obstet Gynecol. 2002;187:1337-42.

24. Sarig G, Younis JS, Hoffman R et al. Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. Fertil Steril. 2002;77:342-7.

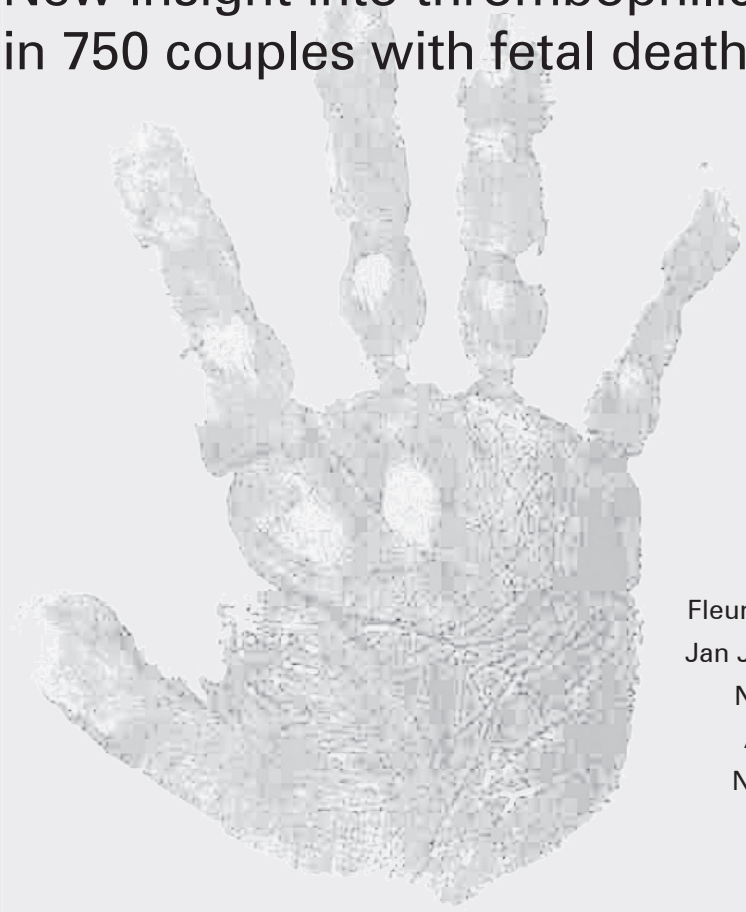
25. Fretts RC. Etiology and prevention of stillbirth. Am J Obstet Gynecol. 2005;93:1923-35.

26. Stallmach T, Hebisch G. Placental pathology: its impact on explaining prenatal and perinatal death. Virchows Arch. 2004;445:9-16.

27. Isermann B, Sood R, Pawlinski R et al. The thrombomodulin-protein C system is essential for the maintenance of pregnancy. Nature. 2003;9:331-337.

Chapter 8

New insight into thrombophilic defects in 750 couples with fetal death



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ABSTRACT

Context

Inherited thrombophilia has been associated with fetal death while thrombophilic defects acquired during pregnancy may further contribute. Their relation to cause of death remains uncertain.

Objective

To determine whether in a cohort of fetal deaths maternal thrombophilic defects, either acquired or inherited, and inherited paternal defects were more prevalent than in the normal population. Furthermore, we assessed the association between these thrombophilic defects and the various fetal death causes.

Design

Multicenter, prospective cohort study from 2002-2006

Setting

50 Dutch secondary and tertiary referral hospitals

Patients

750 couples with singleton ante partum fetal deaths > 20 weeks gestation

Main outcome measures

Antithrombin, protein C, total and free protein S, and von Willebrand factor plasma levels. Mothers were compared to gestational age-matched healthy pregnant women and fathers to healthy men. Prevalence of factor V Leiden, prothrombin G20210A mutation, and lupus anticoagulant were compared to the normal population. A panel classified death cause.

Results

More women with fetal death had decreased antithrombin (16.8%, $p<0.0001$) and protein C (4.0%, $p=0.03$) and increased von Willebrand factor (15.5%, $p<0.0001$) plasma levels than pregnant women (2.5%). However, compared to normal ranges in the non-pregnant population, we only saw more women with increased von Willebrand factor (12.4%, $p<0.0001$). More fathers had decreased free protein S (6.3%, $p<0.0001$) and elevated von Willebrand factor (12.1%, $p<0.0001$) than healthy men (2.5%). Prevalence of inherited thrombophilias was no higher in couples with fetal death than in the population. Neither inherited nor acquired maternal or paternal thrombophilic defects were associated with main cause of death, but of placental causes abruption and infarction were associated with acquired maternal defects.

Conclusions

Except for von Willebrand factor and paternal free protein S, acquired and inherited thrombophilic defects were not associated with fetal death. Acquired maternal thrombophilic defects were only associated with death causes abruption and infarction. Routine thrombophilia testing after fetal death is not advised.

INTRODUCTION

About 1 in 200 pregnancies ends in stillbirth due to a range of causes and conditions.[1] Maternally inherited thrombophilic defects are inconsistently recognized as risk factors for pregnancy complications such as preeclampsia, placental abruption, growth restriction and stillbirth,[2,3] while paternal thrombophilic factors can also be transferred to the fetus and placenta.[4] Many studies have addressed the association between inherited thrombophilic deficiencies and late fetal loss in families with deficiencies, whereas case-control and cohort studies have studied women with fetal loss who were tested for inherited thrombophilia after birth. In reviews a higher risk for fetal loss was observed for antithrombin and protein S deficiency, factor V Leiden, the prothrombin 20210A mutation, and anticardiolipin antibodies.[5-7]

The pathophysiology of late fetal loss associated with inherited thrombophilia was presumed to be placental thrombosis, either in the maternal or fetal circulation of the placenta leading to placental infarction and placental insufficiency.[8] This hypothesis has been difficult to prove due to the small numbers involved in previous studies, while placental insufficiency was often poorly defined and not related to cause of death.[9-12] Pregnancy is a hypercoagulable state due to acquired thrombophilic defects exposing women to a higher risk of thrombosis.[13] In combination with pre-existing inherited thrombophilic defects, it may increase the risk of fetal death. Little is known about the contribution of acquired thrombophilic defects to the hypercoagulable state and fetal death as women in previous studies were only tested for thrombophilia several weeks after delivery.

The objective of our study was to assess whether in a cohort of women with intrauterine fetal death (IUFD), thrombophilic defects, either acquired or inherited, and inherited defects in fathers were more prevalent than in the normal population. Furthermore, we assessed the association between these thrombophilic defects and the various IUFD causes within our cohort.

METHODS

In 2002 we initiated a prospective IUFD cohort study in 50 Dutch secondary and tertiary referral hospitals, serving rural as well as urban populations. Inclusion criteria were singleton IUFD diagnosed ante partum (heart beat ceased before labor), after 20 weeks of gestation calculated from the last menstrual period and confirmed by ultrasonography. Terminations were excluded. The study was approved by the review boards of all the participating hospitals and written informed consent was obtained from participants. Data was collected for each

IUFD, including medical and obstetric history, maternal and fetal characteristics, and pregnancy and birth details. Our diagnostic work-up protocol was based on currently used local Dutch protocols and included: maternal blood tests including full blood count, chemistry and viral serology; coagulation tests for couples performed centrally in the laboratory in Groningen; fetal blood tests including viral serology; microbiological cultures from the mother, fetus and placenta; autopsy; placental examination; and cytogenetic analysis. Ethnic origin was one of the studied characteristics of the study population and classified by the investigator as: Caucasian including Mediterranean groups, African (Negro), Eastern or Other.

Coagulation tests

In women with IUFD, plasma levels of antithrombin (AT), protein C activity (PC), total and free protein S antigen (TPS, FPS), and von Willebrand factor (VWF) were measured in samples collected on induction of labor. These plasma levels were compared to plasma reference values in 110 healthy pregnant women of comparable gestational age recruited from our obstetrics department, after informed consent. Healthy pregnant women were tested at four intervals of gestation: 12-16 weeks, 28-32 weeks, in an early stage of labor (> 34 weeks) and 5-7 weeks postpartum. Women were excluded if they had an individual or family history of venous thromboembolism, known thrombophilia, complications in past pregnancies or the present one, or if they used medication. Reference plasma values were determined for all cases for gestational age periods from 20-27 weeks, 27-34 weeks and >34 weeks (in an early stage of labor). Values at 20-27 weeks gestation were obtained by linear estimation from values at 12-16 weeks and 28-32 weeks for each individual. Plasma levels of AT, PC, TPS and FPS in women with IUFD were defined as abnormal if they were below 2.5% of the values in healthy pregnant women at comparable gestational age, or above 97.5% for VWF.

We also measured plasma levels of AT, PC, TPS, FPS and VWF in the fathers and we determined normal plasma ranges in 393 healthy men. Abnormal plasma levels were defined as levels below the lower limit of the normal ranges: AT <74 IU/dL; PC <64 IU/dL; TPS <67 IU/dL and FPS <65 IU/dL. VWF levels >150 IU/dL were increased. All these abnormal plasma levels will be referred to as 'thrombophilic defects'.

In men and women, factor V Leiden (FVL) and the prothrombin G20210A mutation (PTG20210A) were determined by polymerase chain reactions. Lupus anticoagulant was determined as previously described.[14] Prevalence of these thrombophilias was compared to prevalence in the normal population. AT (Chromogenix, Mölndal, Sweden) and PC activity (Dade Behring, Marburg, Germany) were measured by chromogenic substrate assays; TPS, FPS and VWF were measured by Enzyme Linked Immuno Sorbent Assay (DAKO, Glostrup, Denmark).

Autopsy and placental examination

Autopsies and placental examinations (including histology) were performed by pathologists in the participating hospitals according to guidelines published by the Royal College of Obstetricians and Gynaecologists and the Royal College of Pathologists[15] and the College of American Pathologists.[16,17] Fetal growth percentiles for birth weight by gestational age at time of diagnosis of IUFD were calculated according to Kloosterman's growth charts.[18] Small for gestational age (SGA) was defined as birth weight < 10th percentile.

Adjudication of cause of death

Cause of fetal death was classified by a multidisciplinary panel according to the Tulip classification,[19] which covers six main causes: congenital anomaly, placental pathology, prematurity/immaturity, infection, other (i.e. maternal diseases, fetal hydrops), or unknown. The cause was classified as unknown if other causes had been excluded. Risk factors such as smoking and preeclampsia were defined as contributing to death.

Causes of deaths due to maternal and fetal placental circulation pathology were *placental abruption*, a clinical diagnosis supported by placental examination; significant *infarction* (the percentage of infarctions in relation to the weight of the placenta was regarded as sufficient to have caused death) in preterm cases, any placental infarction, and in term cases, extensive infarction (> 10%) of the placental area,[20] *fetal thrombotic vasculopathy (FTV)*, the presence of avascular villi (at least one focus of five or more villi), thrombosis in a vessel of the chorionic plate or stem villus, hemorrhagic endovasculitis, or intramural fibrin in a vessel of the chorionic plate or stem villus in the absence of umbilical cord blood flow restriction;[21,22] and *maternal floor infarct (MFI)/massive perivillous fibrin deposition (MPFD)*, extensive perivillous fibrin deposition, either predominantly basally located or diffusely distributed in at least 30% of the parenchyma.[23] Other categories of placental pathology were *placental hypoplasia*, an absolute, too low placenta weight (< 10th percentile) and/or a too low placenta/birth weight ratio,[24] and *other placental pathology,* such as villus immaturity and umbilical cord complications.

Statistics

Categorical variables were expressed as counts and percentages, and continuous data as means with standard deviation or median and ranges, with exact 95% confidence intervals (CI) given when appropriate. Differences between groups for categorical data were evaluated by the Fisher exact test or Chi-square test. For continuous variables, we used the Student t test or Mann-Whitney U test, depending on the normality of data. A two-tailed p-value <0.05 was considered

to indicate statistical significance. Statistical analyses were performed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

From 2002 to 2006, we enrolled 750 couples. Coagulation tests were performed in 714 (95.2%) women and 664 (88.5%) men. Autopsies were performed in 525 (70.0%) fetal deaths and placental examinations in 736 (98.1%). The characteristics of men and women with at least one thrombophilic defect (22.4% and 37.4%, respectively) and those without such defects are shown in Table 1. A personal history of venous thromboembolism and known thrombophilia, hypertension-related disease and anticoagulant thromboprophylaxis during pregnancy were observed more often in women with a thrombophilic defect. In this subgroup median age at pregnancy, mean gestational age at delivery (28.4 weeks versus 34.4 weeks) and median birth weight were lower, while it also contained more SGA babies. Men with at least one thrombophilic defect were older than men without defects.

Compared to reference values in non-pregnant women, the majority of women with IUFD and healthy pregnant women had a tendency to higher AT levels, no difference in PC levels, a tendency to lower TPS, lower FPS and higher VWF levels (Figure 1). Compared to healthy pregnant women, women with IUFD had lower levels of AT and higher levels of VWF up to 34 weeks of gestation.

Women with IUFD more often had significantly decreased levels of AT (16.8%) and PC (4.0%), and increased VWF levels (15.5%) compared to pregnant women (2.5%, Table 2). Decreased FPS levels were less common (0.9%). However, when compared to plasma levels in the normal, non-pregnant population, decreased levels of AT (3.7%, $p=0.07$) and PC (2.1%, $p=0.62$) were not observed more often, in contrast to increased VWF levels (87.6%, $p<0.0001$) that were still observed more often in women with IUFD. The prevalence of FVL and PTG20210A in women with IUFD was comparable to the normal population, whereas lupus anticoagulant (1.4%) was observed less frequently than expected. Men in the IUFD group more often had decreased FPS plasma levels (6.3%) and elevated VWF levels (12.1%) compared to healthy men (2.5%).

Causes of death were placental pathology (64.9%), congenital anomaly (5.3%), infection (1.9%), other (4.8%), or unknown (23.1%: 15.9% despite thorough investigation and 7.2% due to insufficient information). Table 3 presents thrombophilic defects found in women with IUFD (n=750) and their partners in relation to cause of death. A thrombophilic defect was seen in 39.1% of women with a placental cause versus 34.1% with a non-placental cause. Overall, in both men and women, none of the separate thrombophilic defects were associated with

Table 1: Characteristics of the study population

Thrombophilic defect	**none n=447**	**at least 1 n=267**	**p**
Women	62.6%	37.4%	
Family history			
Venous thromboembolism (1st degree)	4.9	8.5	0.07
Known hereditary thrombophilia	1.5	4.2	0.06
Personal History			
Venous thromboembolism	0.9	4.9	0.001
Known hereditary thrombophilia	0	2.0	0.006
Previous IUFD	2.7	3.8	0.50
Recurrent early fetal loss	6.3	5.6	0.87
Age, median (range), years	32 (18-46)	30 (18-46)	0.005
Ethnic origin			
Caucasian*	88.4	86.1	0.31
African (Negro)	2.7	5.2	
Eastern	4.3	3.7	
Other	4.7	4.9	
Pregnancy			
Nulliparous	49.7	56.9	0.20
Primiparous	21.9	18.4	
Multiparous	28.4	24.7	
Hypertension-related disease	11.0	25.8	< 0.001
Diabetes-related disease	4.1	3.4	0.84
Smoking	23.3	25.9	0.83
Anticoagulant thromboprophylaxis	1.6	4.5	0.028
Current IUFD			
Gestational age, mean (SD) weeks	34.4 (6.3)	28.4 (5.7)	<0.001
Birth weight, median (range), grams	2000 (40-4630)	810 (12-4425)	<0.001
Small for gestational age†	28.8	43.0	<0.001
Time lap diagnosis and birth, median (range), days	2 (0-40)	2 (0-23)	0.27
Thrombophilic defect	**none n=515**	**at least 1 n=149**	**p**
Men	77.6%	22.4%	
Family history			
Venous thromboembolism (1st degree)	4.4	6.9	0.26
Known hereditary thrombophilia	0.9	2.3	0.19
Personal History			
Venous thromboembolism	0.4	0.7	0.53
Known hereditary thrombophilia	0.2	0.8	0.40
Age, median (range), years	34 (18-61)	35 (19-60)	0.01
Current IUFD			
Gestational age, mean (SD), weeks	31.6 (6.3)	31.7 (6.7)	0.74
Birth weight, median (range), grams	1485 (12-4560)	1395 (51-4630)	0.98
Small for gestational age†	33.5	35.3	0.74

results are given in % unless otherwise indicated, *including Mediterranean groups †according to Kloosterman's growth charts18 which commence at 25 weeks of gestation

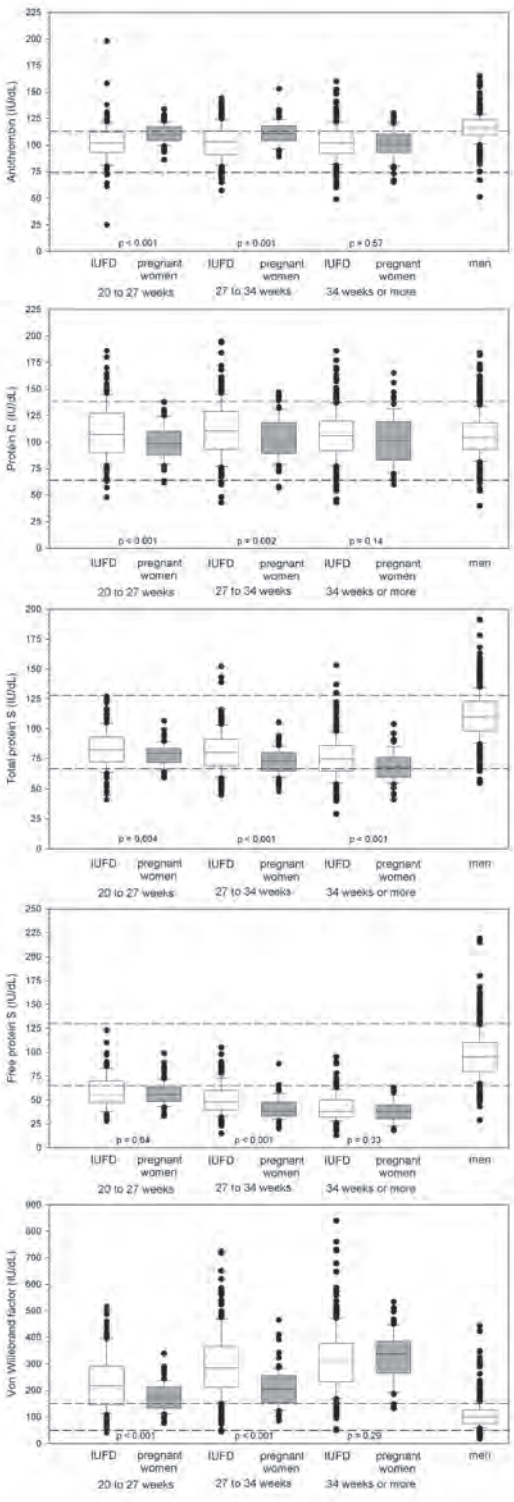

Figure 1. Plasma levels of natural anticoagulant proteins and von Willebrand factor in women with intrauterine fetal death (IUFD), healthy pregnant women and male partners of women with IUFD compared to reference values in the normal non-pregnant population (dotted line).

placental versus non-placental causes. Decreased maternal TPS plasma levels were more often associated with non-placental causes and elevated maternal VWF levels were associated with "other cause of death". In addition, when we considered the 267 women with a thrombophilic defect, 182 (68%) had an IUFD due to a placental cause (39.1% of the 465 women with a placental cause). This was comparable to the group of women without thrombophilic defects (283/447, 63%) (p=0.20). Of the 149 men with a thrombophilic defect, 94 (63%) were in the IUFD group with placental causes (21.7% of 434 men overall with a placental cause), which is comparable to 340/515 men (66%, p=0.56) without a defect. No association with placental causes was observed in couples where there was both a maternal and a paternal thrombophilic defect (32/53; 60% versus 199/317; 63%, p=0.19) versus couples without a defect.

Analysis of placental causes of death showed that death was due to infarction in 99/182 (54%) and abruption in 30/182 (16%) women with a thrombophilic defect (Table 4). Compared to all the different placental causes, abruption was more frequently associated with decreased levels of AT (40.8%, p<0.001), PC (20.4%, p<0.001), and TPS (10.2%, p<0.001) and increased VWF levels (18.4%, p=0.03); infarction was associated with decreased AT (26.1%, p<0.001) and elevated VWF (28.4%, p<0.001) levels and lupus anticoagulant (2.4%, p=0.04); and MFI/MPFD with elevated VWF (28.6%, p<0.001). Abruption was seen significantly more often in women with abnormal plasma levels of AT, PC and TPS compared to infarction. Overall, of placental causes, abruption and infarction were most frequently observed in women with thrombophilic defects (p<0.0001).

DISCUSSION

Our IUFD cohort study was primarily set up to evaluate valuable diagnostics to determine cause of fetal death. Here we addressed the contribution of thrombophilic defects acquired during pregnancy to fetal death, rather than that of inherited thrombophilia. Thrombophilia testing was therefore performed at induction of labor and protein levels in women with fetal death were compared to healthy pregnant women of comparable gestational age. We defined protein levels as potential risk factors for thrombosis in pregnancy (i.e. as thrombophilic defects) when they were < 2.5 percentile in healthy pregnant women for AT, PC and PS, and > 97.5 percentile for VWF. Testing for thrombophilia after fetal death may be useful in clinical practice if the results can be used to prevent recurrent fetal loss. Our data provide no support for routine testing of inherited or acquired thrombophilic defects after fetal death, although acquired defects may play a role in deaths caused by abruption or infarction.

Table 2. Prevalence of thrombophilic defects in couples with intrauterine fetal death (IUFD)

Plasma levels abnormal	Healthy pregnant women#	Women IUFD % (n tested)	p
Antithrombin ↓	2.5%	16.8 (702)	<0.0001
		CI: 14.1-19.8	
Protein C ↓	2.5%	4.0 (708)	0.03
		CI: 2.6-5.7	
Total protein S ↓	2.5%	3.0 (707)	0.48
		CI: 1.9-4.5	
Free protein S ↓*	2.5%	0.9 (699)	0.003
		CI: 0.3-1.9	
VWF↑†	2.5%	15.5 (702)	<0.0001
		CI: 12.9-18.4	
	Prevalence normal population		
Factor V Leiden	5%	6.7 (689)	0.06
		CI: 4.9-8.8	
Heterozygous		6.4	4.0
Homozygous		0.3	0.2
Prothrombin G20210A	3%	3.0 (691)	1.0
		CI: 1.9-4.6	
Heterozygous		3.0	0.9
Homozygous		-	-
Lupus anticoagulant	3%	1.4 (646)	0.01
		CI: 0.6-2.6	

#Reference values, *Free protein S↓ but normal total protein S, †VWF: Von Willebrand Factor, ‡n.a: not applicable

Overall, in women with fetal death, levels of AT and PC remained within the normal ranges for non-pregnant women. These levels are not related to a greater risk for thrombosis in non-pregnant women, but cut-off levels for fetal loss in pregnant women may differ. On the other hand, these proteins may contribute to fetal loss through mechanisms other than their anticoagulant properties, for example cell protection, inhibition of apoptosis of trophoblast cells, and anti-inflammatory effects.[25] This assumption was supported by our finding that IUFD was diagnosed at earlier gestational age and with more SGA fetuses in women with these thrombophilic defects. However, the higher rate of hypertension-related disease in this group could also account for this with the thrombophilia being an epiphenomenon. Decreased levels of PS and increased levels of VWF in most healthy pregnant women, compared to reference values in non-pregnant women, also suggested different cut-off levels in pregnancy.

	Healthy men#	Men IUFD % (n tested)	p
	2.5%	0.3 (655)	<0.0001
		CI: 0.04-1.1	
	2.5%	0.6 (661)	<0.0001
		CI: 0.2-1.5	
	2.5%	0.5 (659)	<0.0001
		CI: 0.1-1.3	
	2.5%	6.3 (656)	<0.0001
		CI: 4.5-8.4	
	2.5%	12.1 (659)	<0.0001
		CI: 9.7-14.9	
	Prevalence normal population		
	5%	4.2 (642)	0.41
		CI: 2.8-6.1	
	3%	0.9 (642)	<0.0001
		CI: 0.3-2.0	
	3%	0 (105)	n.a.‡
		CI: 0-3.5	

Significantly increased maternal VWF plasma levels were observed in our IUFD group compared to healthy pregnant women VWF levels increase during normal pregnancy,[13] but it is unknown when these levels become pathological. VWF activity was found to be higher in women with early miscarriage than in controls.[26] We speculated that our results could be related to non-O blood type[27] or an acute phase response: non-O blood type was indeed associated with higher VWF levels, whereas C-reactive protein and fibrinogen were not (data not shown).

Abnormal paternal plasma levels of FPS and VWF were observed in the IUFD group. Others reported no difference in fetal mortality in women with partners with and without inherited thrombophilia.[28] In contrast, a doubled prevalence (60%) of numerous thrombophilic defects in partners of women with a history of perinatal mortality versus controls (30%) was reported.[29]

Table 3. Thrombophilic defects in couples with intrauterine fetal death (750) in relation to cause of death

Abnormal plasma levels		Placental causes	Non Placental causes		
			Congenital	Infection	Other
		487	40	14	36
Antithrombin ↓	women	18.5 (459)	13.9 (36)	21.4 (14)	18.8 (32)
	men	0.5 (428)	0 (36)	0 (11)	0 (32)
Protein C ↓	women	4.1 (462)	0 (38)	21.4 (14)	3.1 (32)
	men	0.5 (432)	0 (36)	0 (12)	0 (32)
Total protein S ↓	women	2.0 (461)	2.6 (38)	7.1 (14)	3.1 (32)
	men	0.2 (430)	0 (36)	0 (12)	0 (32)
Free protein S ↓†	women	0.9 (456)	0 (38)	0 (14)	0 (32)
	men	4.9 (430)	5.9 (34)	8.3 (12)	12.5 (32)
VWF↑‡	women	17.1 (457)	7.9 (38)	14.3 (14)	34.4 (32)
	men	12.6 (429)	11.1 (36)	25.0 (12)	9.4 (32)
Factor V Leiden	women	7.6 (447)	0 (37)	21.4 (14)	3.1 (33)
	men	4.5 (419)	5.7 (35)	0 (12)	3.1 (32)
Prothrombin	women	3.4 (448)	0 (37)	7.1 (14)	0 (33)
G20210A	men	1.0 (419)	0 (37)	0 (12)	0 (32)
Lupus	women	1.0 (422)	3.0 (33)	7.1 (14)	6.7 (30)
anticoagulant	men	n.a.			
Any defect	women	39.1 (465)	21.1 (38)	64.3 (14)	51.5 (33)
	men	21.7 (434)	22.2 (36)	33.3 (12)	25.0 (32)
	couples	7.4 (432)	5.6 (36)	16.7 (12)	12.5 (32)

percentages (n tested) are given, *p-value for comparison of placental versus non-placental causes, †Free protein S ↓ but normal total protein S, ‡VWF: Von Willebrand Factor

Overall, FVL, PTG20210A and lupus anticoagulant were not associated with fetal death. This is in contrast with earlier studies that reported relative risks for late fetal loss of 2.1-3.3 for maternal FVL, 2.3-3.0 for maternal PTG20210A, and 2.4 for maternal lupus anticoagulant.[6,7,30] Variation in population characteristics could explain these differences.

Trophoblast invasion of the maternal uterine circulation, spiral artery remodeling and maintenance of blood fluidity in the intervillous space require a balance between pro-thrombotic and anti-thrombotic forces. Most deaths in our study were caused by placental pathology. Overall, none of the maternal and paternal thrombophilic defects were related to a placental cause, nor for couples with double thrombophilic defects. Analysis of various placental causes of fetal death showed that thrombophilic defects were only associated with placental abruption and infarction. Abruption was associated with decreased levels of AT, PC and TPS and increased VWF levels, infarction with decreased AT levels, increased VWF levels, and lupus anticoagulant. Furthermore, both pathologies were

	Unknown	Total	
	173	263	p*
	11.8 (161)	13.6 (243)	0.11
	0 (148)	0 (227)	0.55
	3.1 (162)	3.7 (246)	0.84
	1.3 (149)	0.9 (229)	0.61
	5.6 (162)	4.9 (246)	0.04
	1.3 (149)	0.9 (229)	0.28
	1.3 (159)	0.8 (243)	1.0
	8.8 (148)	8.9 (226)	0.06
	9.3 (161)	12.7 (245)	0.13
	10.7 (150)	11.3 (230)	0.71
	5.1 (158)	5.0 (242)	0.20
	2.8 (144)	3.1 (223)	0.41
	3.1 (159)	2.5 (243)	0.65
	1.4 (144)	0.9 (223)	1.0
	0.7 (147)	2.2 (224)	0.29
	31.1 (164)	34.1 (249)	0.20
	23.3 (150)	23.9 (230)	0.56
	8.7 (149)	9.1 (229)	0.19

associated with combined thrombophilic defects and thrombophilic defects in fathers (decreased FPS, increased VWF levels). Our results suggest that acquired thrombophilic defects may play a role in deaths caused by abruption or infarction, which represent one-third of fetal deaths.

Measuring protein levels at the start of induction in women with fetal death might have influenced our results, since these were compared to healthy pregnant women at comparable gestational age. Such an effect seems less likely after comparing the results in the various subgroups. The median time between diagnosis of IUFD and birth was two days, which makes it unlikely that dead fetus syndrome played a role.[31] Placental abruption might be the cause rather than the result of associated thrombophilic defects due to disseminated intravascular coagulation (DIC). Similarly, hypertension-related disease may cause changes in protein levels as a result of impaired liver function. To address possible confounding factors, we performed two extra subgroup analyses. Firstly, we excluded women who might have had DIC due to abruption and, secondly, women with hypertension-related

Table 4. Thrombophilic defects in 487 women with intrauterine fetal death due to placental pathology

Abnormal plasma levels	Abruption	Infarction	FTV*
	52	197	2
Antithrombin ↓	40.8 (49)	26.1 (184)	0 (2)
Protein C ↓	20.4 (49)	3.3 (184)	0 (2)
Total protein S ↓	10.2 (49)	1.7 (184)	0 (2)
Free protein S ↓[¶]	4.1 (49)	0.6 (183)	0 (2)
VWF↑[§]	18.4 (49)	28.4 (183)	0 (2)
Factor V Leiden	4.2 (48)	8.0 (176)	0 (2)
Prothrombin G20210A	6.3 (48)	3.4 (177)	0 (2)
Lupus anticoagulant	0 (48)	2.4 (167)	0 (1)
Any defect women	61.2 (49)	52.9 (187)	0 (2)
Any defect couples	15.2 (46)	9.8 (173)	0 (2)

percentages (n tested) are given, *FTV: Fetal thrombotic vasculopathy, [†]MFI: Maternal floor infarct/ MPFD: Massive perivillous fibrin deposition ‡combination groups 1 to 4: a combination of one of the following causes of death: abruption, infarction, FTV and MFI/MPFD [#]p-value indicates differences between all subgroups, [¶]Free protein S ↓ but normal total protein S, [§]VWF: Von Willebrand Factor

disease. The results were similar to our overall analyses, indicating limited, if any, confounding (data not shown).

The need for routine testing of thrombophilic defects after fetal death is not supported by our results, except in women with a family history of hereditary thrombophilia or a personal history of venous thromboembolism and IUFD, in whom testing could help prevent further maternal venous thromboembolisms.[5] Testing for abnormal levels of AT, PC, TPS or VWF may yield valuable predictors for a subgroup at risk for fetal death caused by abruption or infarction. This aspect should be addressed in future studies.

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MFI/MPFD† 7	Combination group 1 to 4‡ 30	Hypoplasia 90	Other Placental 109	p#
14.3 (7)	7.1 (28)	9.3 (86)	5.8 (103)	<0.0001
0 (7)	0 (30)	2.3 (87)	1.0 (103)	<0.0001
0 (7)	0 (30)	1.2 (87)	0 (102)	0.002
0 (7)	0 (29)	0 (87)	1.0 (99)	0.31
28.6 (7)	10.7 (28)	12.8 (86)	1.0 (102)	<0.0001
0 (7)	6.7 (28)	3.6 (83)	12.9 (101)	0.28
0 (7)	0 (30)	3.6 (83)	3.0 (101)	0.85
0 (6)	0 (26)	0 (79)	0 (95)	0.41
42.9 (7)	23.3 (30)	25.3 (87)	20.4 (103)	<0.0001
0 (7)	0 (29)	5.1 (79)	4.2 (96)	0.008

REFERENCES

1. Silver RM. Fetal death. Obstet Gynecol. 2007;109:153-167.
2. Infante-Rivard C, Rivard GE, Yotov WV et al. Absence of association of thrombophilia polymorphisms with intrauterine growth restriction. N Engl J Med. 2002;347:19-25.
3. Kupferminc MJ, Eldor A, Steinman N et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med. 1999;340:9-13.
4. Khong TY, Hague WM. Biparental contribution to fetal thrombophilia in discordant twin intrauterine growth restriction. Am J Obstet Gynecol. 2001;185:244-245.
5. Middeldorp S. Thrombophilia and pregnancy complications: cause or association? J Thromb Haemost. 2007;5 Suppl 1:276-282.
6. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet. 2003;361:901-908.
7. Robertson L, Wu O, Langhorne P et al. Thrombophilia in pregnancy: a systematic review. Br J Haematol. 2006;132:171-196.
8. Redline RW. Thrombophilia and placental pathology. Clin Obstet Gynecol. 2006;49:885-894.
9. Alonso A, Soto I, Urgelles MF, Corte JR, Rodriguez MJ, Pinto CR. Acquired and inherited thrombophilia in women with unexplained fetal losses. Am J Obstet Gynecol. 2002;187:1337-1342.
10. Arias F, Romero R, Joist H, Kraus FT. Thrombophilia: a mechanism of disease in women with adverse pregnancy outcome and thrombotic lesions in the placenta. J Matern Fetal Med. 1998;7:277-286.
11. Morssink LP, Santema JG, Willemse F. Thrombophilia is not associated with an increase in placental abnormalities in women with intra-uterine fetal death. Acta Obstet Gynecol Scand. 2004;83:348-350.

12. Mousa HA, Alfirevic1 Z. Do placental lesions reflect thrombophilia state in women with adverse pregnancy outcome? Hum Reprod. 2000;15:1830-1833.

13. Stirling Y, Woolf L, North WR, Seghatchian MJ, Meade TW. Haemostasis in normal pregnancy. Thromb Haemost. 1984;52:176-182.

14. Exner T, Triplett DA, Taberner D, Machin SJ. Guidelines for testing and revised criteria for lupus anticoagulants. SSC Subcommittee for the Standardization of Lupus Anticoagulants. Thromb Haemost. 1991;65:320-322.

15. RCOG. Fetal and perinatal pathology. Report of a joint working party. RCOG. 2001. London.

16. Bove KE. Practice guidelines for autopsy pathology: the perinatal and pediatric autopsy. Autopsy Committee of the College of American Pathologists. Arch Pathol Lab Med. 1997;121:368-376.

17. Langston C, Kaplan C, Macpherson T et al. Practice guideline for examination of the placenta: developed by the Placental Pathology Practice Guideline Development Task Force of the College of American Pathologists. Arch Pathol Lab Med. 1997;121:449-476.

18. Kloosterman GJ. On intrauterine growth. The significance of prenatal care. Int J Gynaecol Obstet. 1970;895-912.

19. Korteweg FJ, Gordijn SJ, Timmer A et al. The Tulip classification of perinatal mortality: introduction and multidisciplinary inter-rater agreement. BJOG. 2006;113:393-401.

20. Fox H. Pathology of the Placenta. second ed. London: Saunders Company; 1997.

21. Kraus FT, Acheen VI. Fetal thrombotic vasculopathy in the placenta: cerebral thrombi and infarcts, coagulopathies, and cerebral palsy. Hum Pathol. 1999;30:759-769.

22. Redline RW, Pappin A. Fetal thrombotic vasculopathy: the clinical significance of extensive avascular villi. Hum Pathol. 1995;26:80-85.

23. Katzman PJ, Genest DR. Maternal floor infarction and massive perivillous fibrin deposition: histological definitions, association with intrauterine fetal growth restriction, and risk of recurrence. Pediatr Dev Pathol. 2002;5:159-164.

24. Pinar H, Sung CJ, Oyer CE, Singer DB. Reference values for singleton and twin placental weights. Pediatr Pathol Lab Med. 1996;16:901-907.

25. Isermann B, Sood R, Pawlinski R et al. The thrombomodulin-protein C system is essential for the maintenance of pregnancy. Nat Med. 2003;9:331-337.

26. Marietta M, Facchinetti F, Sgarbi L et al. Elevated plasma levels of factor VIII in women with early recurrent miscarriage. J Thromb Haemost. 2003;1:2536-2539.

27. Kamphuisen PW, Lensen R, Houwing-Duistermaat JJ et al. Heritability of elevated factor VIII antigen levels in factor V Leiden families with thrombophilia. Br J Haematol. 2000;109:519-522.

28. Preston FE, Rosendaal FR, Walker ID et al. Increased fetal loss in women with heritable thrombophilia. Lancet. 1996;348:913-916.

29. de Galan-Roosen AE, Kuijpers JC, Rosendaal FR et al. Maternal and paternal thrombophilia: risk factors for perinatal mortality. BJOG. 2005;112:306-311.

30. Martinelli I, Taioli E, Cetin I et al. Mutations in coagulation factors in women with unexplained late fetal loss. N Engl J Med. 2000;343:1015-1018.

31. Lurie S, Feinstein M, Mamet Y. Disseminated intravascular coagulopathy in pregnancy: thorough comprehension of etiology and management reduces obstetricians' stress. Arch Gynecol Obstet. 2000;263:126-130.

Part III

Fetal death workup guideline

Chapter 9

Evaluation of 1025 fetal deaths; proposal for diagnostic work-up

Submitted

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ABSTRACT

Background

Cause of fetal death is often unexplained. This can be reduced if a systematic diagnostic evaluation is conducted. The optimal workup however is uncertain.

Methods

In a multicenter, prospective cohort study from 2002 to 2008, 1025 couples with fetal death > 20 weeks of gestation were studied. An extensive non-selective diagnostic workup was performed including maternal and fetal blood tests; parental coagulation tests; microbiological cultures; autopsy; placental examination; cytogenetic analysis; radiography and MRI. A multidisciplinary panel classified cause of fetal death and the value of performed diagnostics for allocating the cause.

Findings

Main causes of death were placental pathology (65.2%), congenital anomaly (4.8%), infection (1.8%) and other (5.0%) while in 23.2% cause remained unknown. A Kleihauer-Betke was positive in 11.9% (95% CI 9.8-14.2) of women and IgM antibodies against viruses and Toxoplasmosis in 17.9% (95% CI 15.6-20.5). Inherited and acquired maternal and paternal thrombophilias were not more common than in the normal population. Autopsy and placental examination were abnormal in 51.5% (95% CI 47.7-55.2) and 89.2% (95% CI 87.2-91.1). Prevalence of chromosomal abnormalities was 11.9% (95% CI 8.7-15.7). The most valuable tests for determination of cause of death were placental examination (95.7% 95% CI 94.2-96.8); autopsy (72.6% 95% CI 69.2-75.9) and cytogenetic analysis (29.0% 95% CI 24.4-34.0).

Interpretation

Autopsy, placental examination, cytogenetic analysis and testing for fetal maternal haemorrhage are the basic tests for all fetal deaths. On the basis of these results or specific clinical characteristics further sequential testing is indicated.

INTRODUCTION

Fetal death is a devastating experience for parents and caregivers. A complex chain of events often precedes the fetal death. Health care workers are responsible for providing support to families and for investigating the cause of death. This information can give insight into why death occurred which will aid the parents in the mourning process. Furthermore, it will be of value in determining recurrence risk, counseling and prevention for future pregnancies, audit of the care provided and enables comparison of health care.[1]

Unfortunately the cause of death is reported as unexplained in up to two-thirds of stillbirths.[2,3] In centres that conduct a systematic and well defined evaluation for causes this percentage is lower.[4] However, the optimal workup after fetal death is uncertain, local protocols differ and are often extensive. Consequently, there is discussion about which tests and examinations should be included in an investigative workup to ensure an acceptable chance of determination of the cause of fetal death.

Recent reviews on workup are mostly based on expert opinion.[3,5,6] We therefore aimed to identify valuable tests for determining the cause of fetal death by a multidisciplinary evaluation of diagnostic procedures performed prospectively in a large cohort of couples with fetal death. Our goal was to propose a guideline for workup after fetal death.

MATERIALS AND METHODS

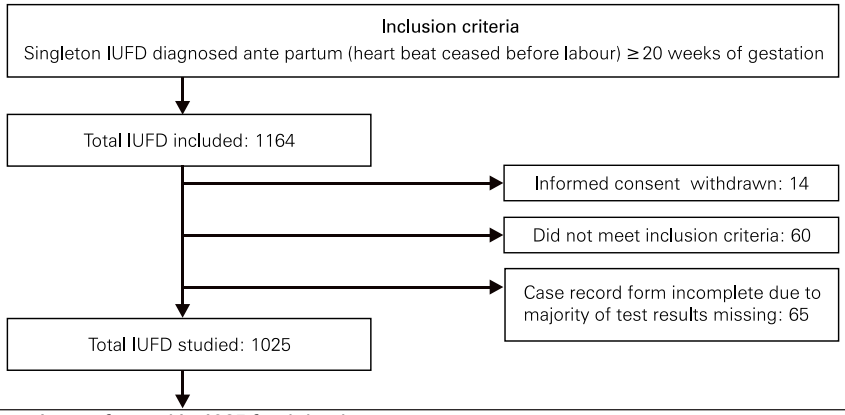
In 2002, we initiated a prospective intrauterine fetal death (IUFD) cohort study in 50 Dutch secondary and tertiary referral hospitals. Inclusion criteria were singleton IUFD diagnosed antepartum after 20 weeks gestation. Pregnancy terminations and intrapartum deaths were excluded. The study was approved by the review boards of all hospitals and informed consent was obtained from all participants.

Diagnostic protocol

Data included medical and obstetric history and details on pregnancy and delivery. Our diagnostic protocol was based on local protocols and diagnostics were included if most hospitals performed these tests after IUFD (Figure 1). Each couple with IUFD was managed the same.

Maternal and fetal blood test results were compared to local laboratory reference values and if exceeding these regarded as abnormal. Maternal and fetal viral serology and microbiological cultures were positive if respectively immunoglobulin levels or culture colonies exceeded the reference values in the local laboratory.

Figure 1. Enrolment of couples with intrauterine fetal death (IUFD) and diagnostics performed after fetal death



Diagnostics performed in 1025 fetal deaths

Prior to delivery after determination of IUFD

Mother

- Blood tests: 67.6-98.6% of individual tests
  Blood type, Rhesus factor, Thrombocytes, Uric Acid, Urea, Creatinine, Aspartate, Alanine, Lactate dehydrogenase (LDH), Bilirubin, Gamma-glutamyl transferase, C-reactive protein, Thyroid-Stimulating Hormone, Free thyroxine (T4), random plasma glucose, HbA1c (glycated hemoglobin), Kleihauer-Betke, Hemoglobin electrophoresis, Anti-nuclear Antibodies, Antibody screening, HIV, random Homocysteine, Anticardiolipin antibodies
- Viral serology: 79.1-94.0% of individual tests
  Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex virus, Parvovirus B19, Syphilis, Hepatitis B-surface-antigen
- Coagulation tests: 81.4-93.7% of individual tests
  plasma levels of Antithrombin, Protein C, total and free Protein S, von Willebrand factor; inherited thrombophilias: Factor V Leiden, Prothrombin G20210A mutation and lupus anticoagulant
- Vaginal-rectal swab: 83.2%

Father

- Coagulation tests: 10.3-87.5% of individual tests
  plasma levels of Antithrombin, Protein C, total and free Protein S, von Willebrand factor; inherited thrombophilias: Factor V Leiden, Prothrombin G20210A mutation and lupus anticoagulant

Fetus

- Cytogenetic analysis; amniocentesis or chorionic villus biopsy: 26.2%

After delivery

Fetus

- Blood tests from umbilical cord: 10.5%
  Blood type, Rhesus factor, Hemoglobin, LDH, Bilirubin, Reticulocytes, Creatine phosphokinase
- Viral serology: 3.6-4.5% of individual tests
  Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex virus, Parvovirus B19, Syphilis
- Fetal ear throat swab: 72.1%
- Autopsy: 68.8%; external fetal examination by expert if no consent for autopsy: 3.2%
- Cytogenetic analysis: 42.1%; tissue testing of the fetus (fetal blood, fascia lata, pericardium, cartilage or skin), umbilical cord or placenta
- MRI: 6.0%
- Radiography: 39.9%

Placenta

- Placental swab between the membranes: 59.7%
- Pathological examination including histopathology: 98.7%

Coagulation tests were performed in couples. Plasma levels collected on induction of labor of antithrombin (AT), protein C activity (PC), total and free protein S antigen (TPS, FPS) and Von Willebrand factor (VWF) were measured and the thrombophilias factor V Leiden (FVL), prothrombin G20210A mutation (PTG20210A) and lupus anticoagulant were determined in our central laboratory. Plasma levels in women and men with IUFD were either compared to levels in 110 healthy pregnant women of comparable gestational age from our hospital or to 393 healthy men. We defined protein levels as potential risk factors for thrombosis when they were < 2.5 percentile for AT, PC, TPS and FPS in healthy pregnant women or healthy men, and > 97.5 percentile for VWF.[7] All these abnormal plasma levels will be referred to as 'thrombophilic defects'. Presence of maternal anticardiolipin antibodies and a random maternal plasma homocysteine (abnormal > 18.5 micromol/l)[8] were tested in local laboratories.

Autopsy and placental examination were performed by surgical and perinatal pathologists in participating hospitals. We urged pathologists to follow the pathology study protocol, based on the guidelines of the Royal College of Obstetricians and Gynaecologists, the Royal College of Pathologists and the College of American Pathologists.[9] Cytogenetic evaluation was performed in genetic centres[10] and radiography and MRI by local radiologists.

Adjudication of cause of death

After individual classification of the cause, mechanism, origin of mechanism and contributing factors of fetal death according to the Tulip classification[9] by an experienced multidisciplinary panel, consensus was reached after discussion. The Tulip classification consists of six main causes: congenital anomaly; placental pathology; prematurity/immaturity; infection; other and unknown.[9] The cause was defined as the initial, demonstrable pathophysiological entity initiating the chain of events that had irreversibly led to death. The mechanism of death was defined as organ failure not compatible with life, initiated by the cause. Origin of mechanism was defined as explanation of the mechanism. Contributing factors such as smoking, obesity and SGA were also identified. Hypertension-related disease during pregnancy included chronic hypertension, pregnancy induced hypertension, preeclampsia, HELLP syndrome and superimposed conditions.[11] Diabetes-related disease during pregnancy included diabetes mellitus type I, II and gestational diabetes with or without medication.[12]

Diagnostics for determination of cause of death were adjudicated valuable if "establishing cause of death" (an abnormal result of a diagnostic test established a cause) or "excluding cause of death" (a result excluded a cause of death when there was a suspected cause). We also registered if a test was "missing for determination of cause of death" (if there was a suspected cause, the result exploring that cause was missing).

Table 1. Cause of death, mechanism of death and origin of mechanism in 1025 intrauterine fetal deaths

Cause of death % (n) / Mechanism of death and origin of mechnism	Total		Cardiocirculatory; congenital heart malformation		Cardiocirculatory; fetal hydrops		Cardiocirculatory; supraventricular tachycardia		Cardiocirculatory; umbilical cord occlusion	
Chromosomal defect; numerical	2.8	(29)	50.0	(2)	20.0	(4)				
Chromosomal defect; structural	0.1	(1)								
Congenital anomaly: syndrome; monogenic	0.1	(1)								
Congenital anomaly: syndrome; other	0.2	(2)								
Congenital anomaly: central nervous system	0.1	(1)			5.0	(1)				
Congenital anomaly: heart and circulatory system	0.4	(4)			10.0	(2)	50.0	(1)		
Congenital anomaly: digestive system	0.1	(1)								
Congenital anomaly: neoplasm	0.3	(3)			5.0	(1)				
Congenital anomaly: other; single organ	0.1	(1)								
Congenital anomaly: other; multiple organ	0,6	(6)	50.0	(2)						
Placenta: placental bed pathology	31.4	(322)								
Placenta: placental pathology; development	18.8	(193)								
Placenta: placental pathology; parenchyma	2.8	(29)								
Placenta: placental pathology; localisation	0.1	(1)								
Placenta: umbilical cord complication	5.7	(58)							98.3	(58)
Placenta: not otherwise specified	6.4	(66)								
Infection: transplancental	1.0	(10)			20.0	(4)				
Infection: ascending	0.8	(8)							1.7	(1)
Other: fetal hydrops of unknown origin	3.3	(34)			35.0	(7)				
Other: maternal disease; diabetus mellitus	0.2	(2)								
Other: maternal disease; hyperthyroidism	0.1	(1)					50,0	(1)		
Other: maternal disease; other	1.3	(13)								
Other: out of the ordinary	0.1	(1)								
Unknown: despite thorough investigation	15.8	(162)			5.0	(1)				
Unknown: important information missing	7.4	(76)								
Total % (n)	100	(1025)	100	(4)	100	(20)	100	(2)	100	(59)

Statistics

Categorical variables were expressed as counts and percentages, and continuous data as means with standard deviation or median and ranges, with exact 95%

	Placental; abruptio placentae		Placental; infarction		Placental; villus immaturity		Placental; hypoplasia		Placental; fetal thrombotic vasculopathy		Placental; massive perivillous fibrindeposition		Infection intrauterine		Other; excessive bleeding		None of the above		Unknown	
			0.7	(2)			6.1	(9)									3.0	(3)	3.2	(9)
																			0.4	(1)
																	1.0	(1)		
																	1.0	(1)	0.4	(1)
																	1.0	(1)		
																			0.4	(1)
																	2.0	(2)		
													7.1	(1)						
																			1.4	(4)
100	(61)	95.9	(257)														4.0	(4)		
				100	(42)	93.2	(137)							15.4	(2)	11.9	(12)		
								100	(4)	100	(7)			76.9	(10)	7.9	(8)		
														7.7	(1)				
																65.3	(66)		
												42.9	(6)						
												50.0	(7)						
		0.4	(1)															9.2	(26)
						0.7	(1)									1.0	(1)		
		2.6	(7)													2.0	(2)	1.4	(4)
																		0.4	(1)
																		56.9	(161)
		0.4	(1)															26.5	(75)
100	(61)	100	(268)	100	(42)	100	(147)	100	(4)	100	(7)	100	(14)	100	(13)	100	(101)	100	(283)

confidence intervals (CI) given when appropriate. Differences between groups were evaluated by the Fisher exact test or Chi-square test for categorical data. A two-tailed p-value <0.05 was considered to indicate statistical significance.

Statistical analyses were performed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

From 2002 to 2008 a total of 1164 couples and their fetal deaths were included, of which 1025 could be studied (Figure1). Investigation into inclusion rates by comparison of death registration yearbooks from participating hospitals yielded an average inclusion of 75% of all IUFDs eligible for the study. Reasons for exclusion were: denied informed consent, a language barrier, logistic problems, and the doctor's reluctance to include women because of an already "known" cause of IUFD at birth. This involved deaths with placental abruption, known

Table 2. Abnormal tests in 1025 intrauterine fetal deaths

Maternal blood tests % abnormal (n tested) 95% CI*	
Thrombocytes < 100 x 103/μl	3.1 (1011) 2.1-4.3
Uric Acid > 0.40 mmol/l	10.6 (956) 8.7-12.7
Urea > 7.5 mmol/l	1.6 (976) 0.9-2.7
Creatinine > 100 umol/l	2.4 (1007) 1.5-3.5
Aspartate > 40 IU/l	8.6 (1001) 6.9-10.5
Alanine > 40 IU/l	7.6 (1006) 6.0-9.4
Lactate dehydrogenase > 250 IU/l	80.6 (988) 78.0-83.0
Bilirubin > 26 umol/l	1.7 (928) 1.0-2.8
Gamma-glutamyl transferase > 40 IU/l	6.5 (914) 5.0-8.3
C-reactive protein > 10 mg/l	51.3 (832) 47.9-54.8
TSH# < 0.4 mE/l	1.6 (936) 0.9-2.6
TSH > 4.0 mE/l	8.9 (936) 7.1-10.9
Free thyroxine < 10.0 pmol/l	13.2 (902) 11.1-15.6
Free thyroxine > 24.0 pmol/l	0.2 (902) 0.03-0.8
Random glucose* 6.1-11.0 mmol/l	19.8 (881) 17.2-22.5
Random glucose ≥ 11.1 mmol/l	0.9 (881) 0.4-1.8
HbA1c (glycated hemoglobin) > 6.0%	7.9 (907) 6.3-9.9
Kleihauer-Betke positive	11.9 (910) 9.8-14.2
Hemoglobin electrophoresis abnormal	2.7 (754) 1.6-4.1
Anti-nuclear antibodies positive	9.4 (693) 7.3-11.8
Antibody screening positive	3.9 (917) 2.8-5.4
HIV positive	0.3 (763) 0.03-0.9

*95% Confidence Interval, #TSH: Thyroid-Stimulating Hormone, *Random plasma glucose, †IgG: immunoglobulin G, ‡IgM: immunoglobulin M, ¶Polymerase chain reaction

chromosomal abnormalities and major congenital anomalies, which resulted in an under-representation of such deaths in our cohort.

Median age of mothers was 30 years (range 17-51 years) and median gestational age at determination of IUFD 32 weeks and 0 days (32^0) (range 20^0-42^4). The distribution of maternal ethnic origin was 87.1% Caucasian, 4.6% African, 3.8% Eastern and 4.5% Other. Of these mothers 52.7% were nulliparous. Median fetal weight was 1528 gram (range 12 to 5410 gram). Of these babies 37.2% were SGA and 10.1% LGA.[13]

The main causes of death were placental pathology (65.2%), congenital anomaly (4.8%), infection (1.8%) and other (5.0%) while in 23.2% the cause remained unknown. This was despite thorough investigation in 15.8%, whereas in 7.4% important information on diagnostics was missing. Subgroups of causes, mechanism and origin of mechanism of death are presented in Table 1. Deaths

Positive viral serology % abnormal (n tested) 95% CI	**Maternal**	**Fetal**
Toxoplasmosis IgG†	23.4 (964) 20.8-26.3	9.8 (41) 2.7-23.1
Toxoplasmosis IgM‡	1.4 (946) 0.7-2.3	0 (42) 0-8.4
Rubella IgG	90.5 (944) 88.4-92.3	75.7 (37) 58.8-88.2
Rubella IgM	3.2 (853) 2.1-4.6	0 (37) 0-9.5
Cytomegalovirus IgG	46.8 (941) 43.5-50.0	42.5 (40) 27.0-59.1
Cytomegalovirus IgM	2.3 (964) 1.4-3.4	0 (46) 0-7.7
Herpes simplex virus IgG	63.3 (811) 59.8-66.6	50.0 (40) 33.8-66.2
Herpes simplex virus IgM	7.9 (826) 6.1-9.9	0 (41) 0-8.6
Parvovirus B19 IgG	62.8 (869) 59.5-66.1	45.0 (40) 29.3-61.5
Parvovirus B19 IgM	2.1 (892) 1.3-3.3	2.3 (43) 0.06-12.3
Syphilis	0.5 (946) 0.2-1.2	2.3 (44) 0.06-12.0
Hepatitis B-surface-antigen	0.5 (934) 0.2-1.2	
Cultures		
Urinary sediment nitrite positive	3.8 (771) 2.5-5.4	
Chlamydia PCR¶	1.6 (706) 0.8-2.8	
Group B Streptococcus	17.9 (853) 15.4-20.7	
Fetal swabs		25.3 (739) 22.2-28.6
Placental swabs		22.8 (612) 19.6-26.4
Other diagnostics		
Autopsy		51.5 (705) 47.4-55.2
Placental examination		89.2 (1012) 87.2-91.1
Cytogenetic analysis		11.9 (362) 8.7-15.7
MRI		30.6 (62) 19.6-43.7
Radiography		7.3 (409) 5.0-10.3

Table 3. Prevalence of thrombophilic defects in 1025 couples with intrauterine fetal death

Plasma levels abnormal	Healthy pregnant women[#]	Women IUFD % (n tested) 95% CI[§]	p
Antithrombin ↓	2.5%	17.1 (952)	<0.0001
		CI: 14.7-19.7	
Protein C ↓	2.5%	4.2 (834)	0.005
		CI: 2.9-5.8	
Total protein S ↓	2.5%	2.9 (960)	0.46
		CI: 2.0-4.2	
Free protein S ↓*	2.5%	1.0 (952)	<0.0001
		CI: 0.4-1.8	
VWF ↑†	2.5%	14.3 (954)	<0.0001
		CI: 12.1-16.6	
	Prevalence normal population		
Factor V Leiden	5%	5.8 (935)	0.31
		CI: 4.4-7.5	
Heterozygous		5.6	
Homozygous		0.2	
Prothrombin G20210A	3%	2.4 (937)	0.28
		CI: 1.5-3.5	
Heterozygous		2.4	
Homozygous		-	
Lupus anticoagulant	3%	1.5 (865)	0.007
		CI: 0.8-2.6	
Random homocysteine	5%	2.9 (733)	0.006
> 18.5 umol/l		CI: 1.8-4.4	
Anticardiolipin	2-10%	5.6 (791)	n.a.
antibodies		CI: 4.1-7.4	

[#]Reference values in healthy pregnant women at comparable gestational age, [§]95% Confidence Interval *Free protein S↓ but normal total protein S, [†]VWF: Von Willebrand factor, [‡]n.a: not applicable

caused by fetal maternal haemorrhage (FMH) of unknown origin with evidence of fetal anaemia confirmed by placental examination and/or autopsy (1.3%) were classified according to the Tulip classification as caused by placental parenchyma pathology (n=10) or placenta not otherwise specified (n=3); both with origin of mechanism excessive bleeding. In 10.6%, FMH was observed as a contributing factor. In 3.3% fetal hydrops of unknown origin was adjudicated as cause of death. Diabetes-related disease was cause of death twice in which maternal diabetic coma resulted in fetal death. In 4.0% diabetes-related disease was a contributing factor. Maternal hyperthyroidism resulted in fetal death only once, in 2.1% thyroid-related disease was a contributing factor. Most deaths in the maternal disease group other (1.3%) were caused by a known antiphospholipid syndrome before

	Healthy men#	Men IUFD % (n tested) 95% CI	p
	2.5%	0.4 (894)	<0.0001
		CI: 0.1-1.1	
	2.5%	0.9 (774)	0.002
		CI: 0.4-1.9	
	2.5%	0.7 (897)	<0.0001
		CI: 0.3-1.5	
	2.5%	6.6 (893)	<0.0001
		CI: 5.1-8.4	
	2.5%	12.4 (896)	<0.0001
		CI: 10.3-14.7	
	Prevalence normal population		
	5%	5.0 (872)	0.99
		CI: 3.7-6.7	
		4.9	
		0.1	
	3%	1.4 (872)	0.003
		CI: 0.7-2.4	
		1.4	
		-	
	3%	0 (106)	n.a.‡
		CI: 0-3.4	

the index pregnancy (0.9%). The most frequent observed maternal disease during pregnancy was hypertension-related disease (15.1%), placental bed pathology was cause in 65.8% of these deaths.

Performance rates for the different diagnostics of our protocol varied from 98.7% for placental examination to 3.2% for external fetal examination by an expert (Figure 1). Abnormal maternal blood tests (Table 2) were observed in 80.6% for lactate dehydrogenase, 51.3% for C-reactive protein, 19.8% for random glucose and a Kleihauer Betke was positive in 11.9%. Increased Thyroid-Stimulating Hormone and/or decreased free thyroxine were observed in respectively 8.9% and 13.2%. Other maternal blood tests were abnormal in 10% or less. Of the women with increased HbA1c (7.9%) 61.8% was not known to have diabetes-related disease. Macrosomia and obesity; known risk factors for diabetes[14] were more prevalent in this group compared to the group with normal HbA1c. Fetal blood

Table 4. Valuable tests in relation to determination of cause in 1025 intrauterine fetal deaths

Diagnostic tests			Kleihauer-Betke			Glucose testing			Hb electro-phoresis			Antibody screening		
Cause of death (n)	Value of diagnostics	Total	determined	excluded	missed	determined	excluded	missed	determined	excluded	missed	determined	excluded	missed
Chromosomal defect; numerical		29												
Chromosomal defect, structural		1												
Congenital anomaly		19												
Placenta: placental bed pathology		322						1						
Placenta: placental pathology; development		193	1		1	2	6	1						
Placenta: placental pathology; parenchyma		29	10				1							
Placenta: placental pathology; localisation		1												
Placenta: umbilical cord complication		58												
Placenta: not otherwise specified		66	2			1	1							
Infection		18					1							
Other: fetal hydrops of unknown origin		34		1						1	33		30	4
Other: maternal disease; diabetes mellitus		2				2								
Other: maternal disease; hyperthyroidism		1												
Other: maternal disease; other		13												
Other: out of the ordinary		1												
Unknown: despite thorough investigation		162		2			2						1	
Unknown: important information missing		76		2	1		3							
Total, n		**1025**	**13**	**5**	**2**	**5**	**14**	**2**	**0**	**1**	**33**	**0**	**31**	**4**
n tested			910			907			754			917		
n not tested					115			118			271			108
Total, % valuable			2.0		1.7	2.1		1.7	0.1		12.2	3.4		3.7

#Anticardiolipin antibodies

tests derived from the umbilical cord were only performed in 10.5% mainly due to the impossibility of drawing (enough) blood after birth.

Of the women tested for Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex virus, Parvovirus B19, Syphilis or Hepatitis B-surface-antigen (HBsAg) 17.9% (95% CI 15.6-20.5) had positive IgM antibody titers to one of these infectious agents or a positive HBsAg (Table 2). In 1.8% intrauterine infection was allocated as cause of death, supported by placental or autopsy findings. These infections were caused by Toxoplasmosis (n=1), Cytomegalovirus (n=1), Parvovirus B19 (n=3), Syphilis (n=2), Listeria (n=1), E.coli (n=1), proteus mirabilis (n=1), group B streptococcus (n=4) and in four cases no specific micro-organism could be detected. Of the fetal ear/throat and placental swabs respectively 25.3% and 22.8% were positive

	Anticardio-lipin AB#			Mother viral serology			Mother swabs			Fetus swabs			Fetus viral serology			Cyto-genetic analysis			Autopsy			Placenta			MRI		
	determined	excluded	missed	determined	excluded	missed	determined	excluded	missed	determined	excluded	missed	determined	excluded	missed	determined	excluded	missed	determined	excluded	missed	determined	excluded	missed	determined	excluded	missed
																29			13	3	1	13	6		1		
																1											
																	4	6	15	2	1	5	3				
	1	1			2										1		20	16		155	44	319					
					1			1			1			1			9	8	1	59	28	193					
																	1	1	5	6	4	28					
																				1		1					
																		1	6	14	6	50		1	1		
					2	1									1		1	5		22	11	66					
				8			3		1	6		1	1						15	1		18					
					31	3		1				1		2	32		15	6		26	8		34				
																				1							
																			1				1				
	7																			10		8	3	1			
																			1			1					
					2	1		1	1		2	1		2			24	19		139	17		153	3		1	
					1	2		1			1	1		2	2		1	25		16	59		66	5			
	8	**1**		**8**	**39**	**7**	**3**	**4**	**2**	**6**	**4**	**4**	**1**	**7**	**36**	**30**	**75**	**87**	**57**	**455**	**179**	**702**	**266**	**10**	**2**	**1**	**0**
	791			964			853			739			46			362			705			1012			62		
			234			61			172			286			979			663			320			13			963
	1.1		0.0	4.9		11.5	0.8		1.2	1.4		1.4	17.4		3.7	29.0		13.1	72.6		55.9	95.7		76.9	4,8		0,0

for one or more microorganisms. Of the positive fetal and placental swabs in respectively 74.9% and 71.4% E.coli or group B streptococcus was detected. Of placentas 12.7% (95% CI 10.7-14.9) showed histological chorioamnionitis without funisitis and only 21.9% and 25.9% of the fetal and placental swabs were positive. Of the maternal vaginal-rectal swabs 17.9% (95% CI 15.4-20.7) was positive for group B streptococcus (Table 2).

In women with IUFD significantly more often decreased plasma levels of AT (17.1%) and PC (4.2%), and increased levels of VWF (14.3%) were observed when compared to healthy pregnant women (Table 3). However, when compared to plasma levels in the normal, non-pregnant population, only abnormal levels of AT (4.2%, p=0.003) and VWF (86.7%, p<0.0001) were more often observed. More men with IUFD had decreased FPS plasma levels (6.6%) and elevated VWF

levels (12.4%) compared to healthy men. Overall, inherited maternal or paternal thrombophilias in couples with IUFD were not more prevalent than in the normal population. This was also concluded for maternal hyperhomocysteinemia or presence of maternal anticardiolipin antibodies. Of the 40 women not known with an antiphospholipid syndrome before the index pregnancy with either lupus anticoagulant and/or anticardiolipin antibodies, 12 IUFDs were caused by placental bed pathology with origin of mechanism infarction, 23 had other causes and five an unknown cause of death.

Abnormal findings were observed in 89.2% of placentas, in 51.5% of autopsies, 30.6% of MRI and 7.3% of radiography. Cytogenetic analysis was performed in 700 IUFDs (68.3%) and a successful result obtained in 362 deaths (51.7%). The prevalence of a chromosomal abnormality in these 362 IUFDs was 11.9% (95% CI 8.7-15.7). Of these 43 chromosomal abnormalities: 37.2% was trisomy 21; 23.3% trisomy 18; 16.3% monosomy (45, X); 4.6% trisomy 13 and in 18.6% other chromosomal abnormalities were found.

The most valuable tests for adjudication of cause of death were placental examination in 95.7% (95% CI 94.2-96.8); autopsy in 72.6% (95% CI 69.2-75.9); and cytogenetic analysis in 29.0% (95% CI 24.4-34.0) (Table 4). The tests not mentioned in Table 4 including coagulation tests and radiography were respectively never or only once allocated as valuable for adjudication of the cause. On the basis of our findings we propose a flowchart guideline for diagnostics after fetal death to determine the cause (Figure 2).

DISCUSSION

A fetal death work-up guideline helps in elucidating cause of fetal death but also aims to prevent unnecessary investigations. There is no international generally accepted diagnostic guideline for fetal death. This study primarily addressed the value of a uniform extensive non-selective protocol of diagnostic tests for the adjudication of the cause in 1025 fetal deaths. This resulted in a proposal for a basic and additional workup guideline. In the cognitive process of making explicit the complex chain of events eventually leading to adjudication of an underlying cause of death during our panel meetings we also assessed risk factors and the clinical setting in which fetal death occurred.[9]

The value of this study lies in the approach in which fetal death was evaluated and the size of the cohort. For classification we used the Tulip classification for perinatal mortality that separates cause, mechanism and risk factors, which are often, mixed.[15] Having strict criteria the Tulip classification system itself influenced our adjudication of causes and judgment if tests were valuable. This study was

not a case-control study because it was not our intention to determine differences between abnormal tests in IUFDs versus liveborns. Some abnormal tests may also be common in live births therefore it is difficult to judge their relevance when found in a cohort of stillbirths. In general, the occurrence of diagnostic test abnormalities in our study was low. In our procedure of classifying the cause all available information including the clinical setting in addition to abnormal test results and other diagnostics were considered.

In accordance with others,[16-18] we cannot overemphasize the value of autopsy (72.6%). Earlier studies also provided evidence that after IUFD routine macroscopic and histological examination of the placenta is a necessary complement to autopsy confirming clinical and/or autopsy findings.[19-21] Placental examination was valuable in 95.7%. Both tests, ideally performed by a perinatal pathologist provide information that is pertinent to nearly every potential cause of fetal death. It is fundamental to provide the pathologist with essential clinical information in guiding appropriate investigation into causes. Further selective diagnostic testing may follow after pathology results and/or suspect clinical characteristics.

Although an autopsy is optimal, earlier studies have acknowledged the value of MRI as an alternative,[22] particularly for cerebral pathology.[23] Our low performance rate of MRI (6.1%) is probably due to our high autopsy rate, clinicians not being familiar with fetal MRI and unavailability of MRI services. MRI was abnormal in 30.6% and radiography in 7.3%. MRI was adjudicated as valuable in only two cases and radiography even less. This is probably associated with inexperience of most radiologists with judging MRI after fetal death. MRI and radiography are advised if recommended by an expert after prenatal ultrasound or external fetal examination with suspected congenital anomalies and especially in the absence of autopsy.

We observed chromosomal abnormalities in 11.9% of our successfully karyotyped IUFDs. For adjudication of the cause cytogenetic analysis was the third most valuable test (29.0%) and is advised for all IUFDs by invasive testing before labour. If no parental consent for invasive testing is given an umbilical cord sample postpartum is second best.[10]

Fetal maternal haemorrhage (FMH) was attributed as cause (1.3%) if there was evidence of fetal anaemia confirmed by placental examination and/or autopsy. Others reported 3% of deaths caused by FMH.[24] We observed FMH in 11.9%, comparable to 8% reported in stillbirths earlier.[25] We would be inclined to advice investigation for FMH only in cases with suspicion. However, to preserve erythrocytes until after pathology results are available is technically impossible. We therefore recommend investigation of FMH in all fetal deaths before induction of labour, to exclude FMH caused by labour itself.

Fetal deaths but also live births have been associated with bacterial, viral and protozoal infections. The most convincing proof of an infectious cause of death

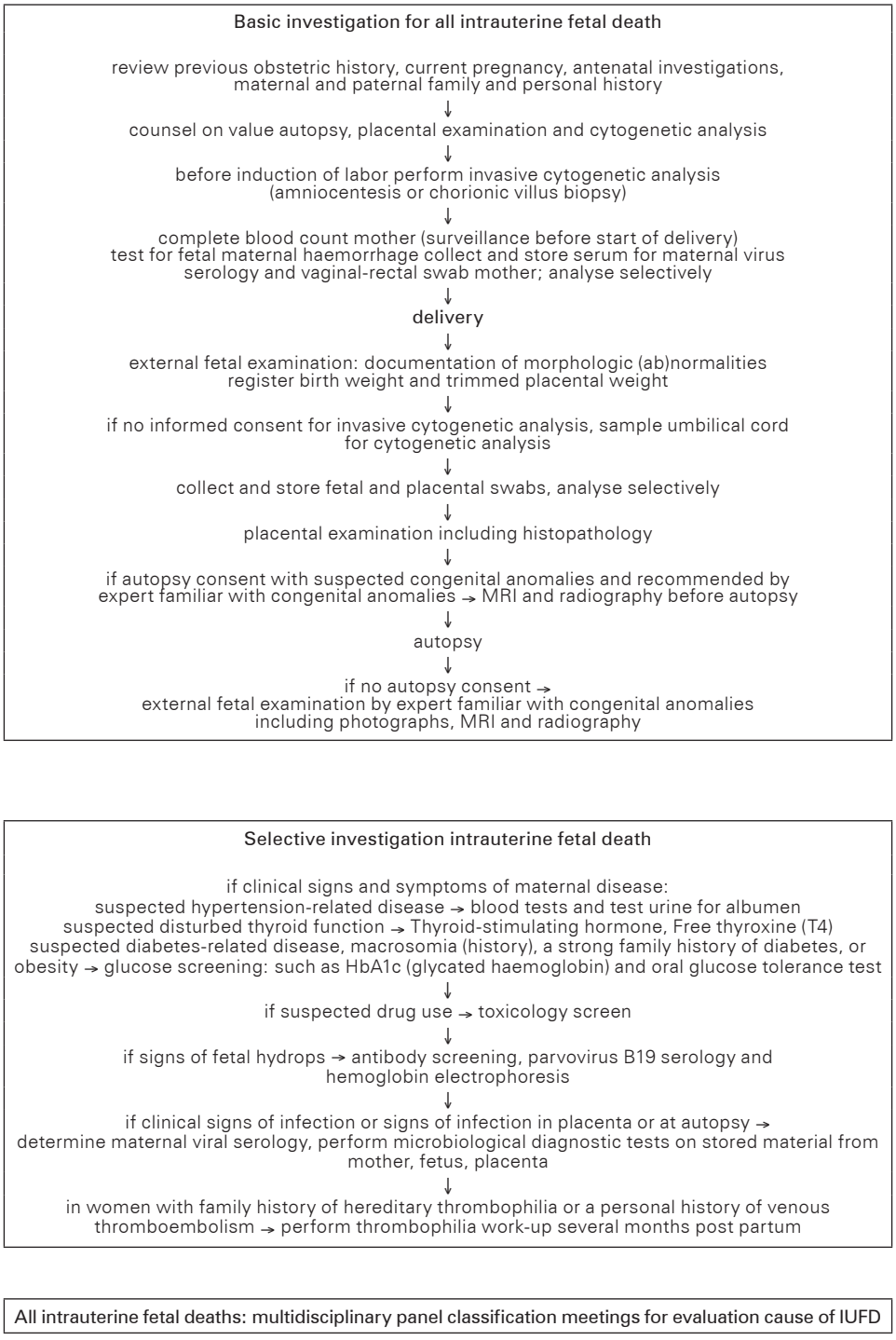
is an autopsy with evidence of organ involvement with an organism potentially able to cause stillbirth and/or histological placental examination with infectious findings.[26] Chorioamnionitis by itself observed in 12.7% should not be considered a cause of stillbirth. TORCH serology and cultures are traditionally advised in the evaluation of stillbirth. In our study placental examination and autopsy were able to support an intrauterine infection as cause in only 1.8%. This is supported by others.[17,27] More infections were reported by Petersson et al, 24% in fetal deaths after 22 weeks gestation, most commonly group B streptococcus.[4] Differences could be explained due to our criteria and the lack of use of molecular diagnostic technology. On the base of our findings we no longer recommend routine screening for infections. We advise to obtain and store blood for maternal virus serology and material for maternal, fetal and placental cultures but to only analyse these in cases with suspicion of a maternal or intrauterine infection. However, in parts of the world with a high prevalence of infectious causes such as syphilis and malaria[28] testing all deaths may well be advisable.

In an earlier publication, we concluded after analysis of 750 fetal deaths that our data provided no evidence for routine testing of inherited or acquired thrombophilic defects after fetal death.[7] This is confirmed by our data of 1025 evaluated fetal deaths. Thrombophilia testing should be considered in women with IUFD and a family history of hereditary thrombophilia or a personal history of venous thromboembolism, to prevent further maternal thromboembolisms.[29]

Overall about 10% of fetal deaths are associated with maternal disease.[30] We observed hypertension and diabetes-related disease during pregnancy in respectively 15.1% and 4.0%. Diabetes-related disease as cause of death was rare. However, an increased HbA1c was observed in 7.9% of which 61.1% were previously unknown, illustrating that un-established gestational diabetes and IUFD needs further scientific investigation. Only one death was caused by thyroid-related disease. Testing for maternal disease in asymptomatic women has been suggested by many,[6] we recommend testing selectively if there is a suspect clinical history or suspect current pregnancy. In cases with fetal hydrops tests to prove red cell alloimmunzation, parvovirus B19 serology and haemoglobin electrophoresis are advised to exclude other causes of death as these were considered valuable diagnostics in these cases.

The clinical implications of our findings are presented in a flowchart for a diagnostic workup guideline to determine cause of fetal death (Figure 2). Autopsy, placental examination and cytogenetic analysis are the base for diagnostic work-up for all fetal deaths. We recommend further individualised sequential testing to avoid unnecessary investigations and positive test results that do not identify the cause of stillbirth and bring on anxiety. Modifications to the guideline, for example testing of HbA1c can be applied in view of risk factor assessment, no autopsy consent,

Figure 2. Guideline flowchart for diagnostic workup to investigate cause of fetal death



endemic differences in causes of fetal death, local preferences, different cultures, technology or financial resources.

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REFERENCES

1. Gardosi J, Kady SM, McGeown P, Francis A, Tonks A. Classification of stillbirth by relevant condition at death (ReCoDe): population based cohort study. BMJ. 2005;331:1113-1117.
2. Fretts RC. Etiology and prevention of stillbirth. Am J Obstet Gynecol. 2005;193:1923-1935.
3. Goldenberg RL, Kirby R, Culhane JF. Stillbirth: a review. J Matern Fetal Neonatal Med. 2004;16:79-94.
4. Petersson K, Bremme K, Bottinga R et al. Diagnostic evaluation of intrauterine fetal deaths in Stockholm 1998-99. Acta Obstet Gynecol Scand. 2002;81:284-292.
5. Silver RM, Varner MW, Reddy U et al. Work-up of stillbirth: a review of the evidence. Am J Obstet Gynecol. 2007;196:433-444.
6. Smith GC, Fretts RC. Stillbirth. Lancet. 2007;370:1715-1725.
7. Korteweg FJ, Erwich JJ, Folkeringa N et al. New insight into thrombophilic defects in 750 couples with fetal death. 2009. Submitted.
8. den Heijer M, Koster T, Blom HJ et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med. 1996;334:759-762.
9. Korteweg FJ, Gordijn SJ, Timmer A et al. The Tulip classification of perinatal mortality: introduction and multidisciplinary inter-rater agreement. BJOG. 2006;113:393-401.
10. Korteweg FJ, Bouman K, Erwich JJ et al. Cytogenetic analysis after evaluation of 750 fetal deaths: proposal for diagnostic workup. Obstet Gynecol. 2008;111:865-874.
11. Brown MA, Lindheimer MD Swiet M de et al. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Association for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy 20, IX-XIV. 2001.
12. Dutch Society of Obstetrics and Gynecology Practice guideline. Diabetes mellitus and pregnancy. 32, 1-6. 2001. Utrecht.
13. Kloosterman GJ. On intrauterine growth. The significance of prenatal care. Int J Gynaecol Obstet. 1970;895-912.
14. Gilmartin AB, Ural SH, Repke JT. Gestational diabetes mellitus. Rev Obstet Gynecol. 2008;1:129-134.
15. Korteweg FJ, Gordijn SJ, Timmer A, Holm JP, Ravise JM, Erwich JJ. A placental cause of intra-uterine fetal death depends on the perinatal mortality classification system used. Placenta. 2008;29:71-80.
16. Gordijn SJ, Erwich JJ, Khong TY. Value of the perinatal autopsy: critique. Pediatr Dev Pathol. 2002;5:480-488.
17. Incerpi MH, Miller DA, Samadi R, Settlage RH, Goodwin TM. Stillbirth evaluation: what tests are needed? Am J Obstet Gynecol. 1998;178:1121-1125.
18. Saller DN, Jr., Lesser KB, Harrel U, Rogers BB, Oyer CE. The clinical utility of the perinatal autopsy. JAMA. 1995;273:663-665.
19. Gordijn SJ, Dahlstrom JE, Khong TY, Ellwood DA. Histopathological examination of the placenta: key issues for pathologists and obstetricians. Pathology. 2008;40:176-179.
20. Korteweg FJ, Erwich JJ, Holm JP et al. **Diverse placental pathologies as the main causes of fetal death**. Obstet Gynecol. 2009;114:809-817.

21. Rayburn W, Sander C, Barr M, Jr., Rygiel R. The stillborn fetus: placental histologic examination in determining a cause. Obstet Gynecol. 1985;65:637-641.

22. Brookes JA, Hall-Craggs MA, Sams VR, Lees WR. Non-invasive perinatal necropsy by magnetic resonance imaging. Lancet. 1996;348:1139-1141.

23. Wright C, Lee RE. Investigating perinatal death: a review of the options when autopsy consent is refused. Arch Dis Child Fetal Neonatal Ed. 2004;89:285-288.

24. Laube DW, Schauberger CW. Fetomaternal bleeding as a cause for "unexplained" fetal death. Obstet Gynecol. 1982;60:649-651.

25. Bohra U, Regan C, O'Connell MP, Geary MP, Kelehan P, Keane DP. The role of investigations for term stillbirths. J Obstet Gynaecol. 2004;24:133-134.

26. Goldenberg RL, Thompson C. The infectious origins of stillbirth. Am J Obstet Gynecol. 2003;189:861-873.

27. Benirschke K, Robb JA. Infectious causes of fetal death. Clin Obstet Gynecol. 1987;30:284-294.

28. McClure EM, Goldenberg RL. Infection and stillbirth. Semin Fetal Neonatal Med. 2009;14:182-189.

29. Middeldorp S. Thrombophilia and pregnancy complications: cause or association? J Thromb Haemost. 2007;5 Suppl 1:276-282.

30. Simpson LL. Maternal medical disease: risk of antepartum fetal death. Semin Perinatol. 2002;26:42-50.

General discussion and future perspectives

As described in this thesis, to gain more insight into causes of intrauterine antepartum fetal death and perinatal mortality as a whole an optimal multidisciplinary classification of cause of death, mechanism, origin of mechanism and contributing factors to death are essential. By improving diagnostic work-up after intrauterine antepartum fetal death, we can enhance the chances of determining the cause of fetal death. By studying occurrence and clinical manifestations of different causes of death the pathophysiology can be further unravelled. The following paragraphs describe aspects of focus and currently ongoing and future research on fetal death, the development of audit of perinatal mortality, consensus regarding diagnostic investigations into causes of fetal death and the development of possible strategies to bring down fetal death rates.

Fetal death

Fetal death is gradually being recognised as a global major health problem. One of the millennium goals of the World Health Organisation (WHO) was "Make Every Mother and Child Count". This project examines the reasons why so many children under five years of age and women in pregnancy, during childbirth or soon after continue to die from causes that are largely preventable. In addition to this project, the WHO considers stillbirth as an indicator for performance of health care service delivery systems. A WHO global goal is to bring down fetal death rates, to raise the issue of this public health tragedy and to secure political commitment for adequate investment. In 2000 the International Stillbirth Alliance (ISA) was introduced, a non-profit coalition of organizations dedicated to understanding the causes and prevention of stillbirth. In the meantime, this organisation has brought together international leading researchers in the field of stillbirth enabling collaboration on different aspect of work and discussion on international consensus regarding definition, classification and diagnostic work-up after fetal death. In order to compare perinatal mortality rates including stillbirth, uniform definitions concerning the perinatal period are needed. Globally these differ. Despite earlier recommendations, definitions within Europe also still differ.[1] Nationally there is now definition consensus; this was accomplished by linking the datasets of the national obstetric registration (LVR 1-2) and the national neonatology registration (LNR). Perinatally related deaths beyond 20 weeks of gestation are defined as stillbirths, early neonatal deaths are deaths up to seven completed days after birth, late neonatal deaths are deaths from eight up to 28 completed days after birth and perinatally related infant deaths are deaths from 29 days up to six completed months after birth during hospital admission from birth onwards.

Raising public awareness is also one of ISA's goals. Regarding this aspect in the United Kingdom, SANDS a large stillbirth and neonatal death charity has recently initiated the public champagne: "Why are 17 babies a day dying and what can be done to halt this national tragedy?" In the Netherlands this public awareness has partly been raised by the results of the Peristat studies,[2,3] revealing that the Netherlands is among the European countries with the highest perinatal mortality rates. However, there is little publicity regarding perinatal mortality compared to the amount of campaigns for instance on lethal traffic accidents, which is half the number of perinatal mortality. In addition, in the Netherlands it is very difficult to acquire money for research in the field of perinatal mortality; there are no fund raising organisations and few grants for this research field.

Classification of cause of perinatal mortality

The presented studies in this thesis addressed the need for a uniform classification system for perinatal mortality to be used in multidisciplinary panel meetings. Our proposed "Tulip classification" aids in classification of underlying cause of death, mechanism, origin of mechanism and contributing factors to perinatal mortality. In comparison to other classification systems, this system studies fetal death by considering all three entities mother, fetus and placenta and their interaction. The Tulip system does not confuse underlying cause of death with mechanism of death, risk factors and clinical manifestations. It has accessible user guidelines with case examples and defined agreements. We acknowledge that this system is ambitious and extensive requiring adequate perinatal mortality input data. However, in a well-developed country as the Netherlands we should be able to meet these quality requirements. The Tulip classification mainly answers the question "why" perinatal mortality occurred. By combining this system with other classification systems in sequence of increasing difficulty our proposed multilayered classification system also answers the questions "when" and "what" concerning perinatal mortality. By using a combination of classification systems this may compensate for shortcomings of individual systems. The high proportion of unexplained stillbirths in developing countries is a major barrier to future improvement in the stillbirth rate. A substantial proportion of unexplained stillbirths are almost certainly a result of inadequate investigation rather than a medical mystery. Classification systems that facilitate storage and retrieval of important information in the understanding of the death are essential. In countries with fewer resources, health care workers are able to classify perinatal deaths in the multilayered classification system to the extent of what is possible.

Multidisciplinary audit groups

For an optimal review and classification of perinatal deaths multidisciplinary audit groups consisting of obstetricians, perinatal pathologists, neonatologists, geneticists and in some cases other specialists are essential. Discussions in these meetings provide the best insight into the pathophysiology of these deaths and may result in an optimal counselling of parents on the outcome. In developing and developed countries, suboptimal factors that contribute to perinatal death have been identified. The EuroNatal study involving ten European countries showed that substandard care was possibly or likely to have contributed to death in about half of the 1619 perinatal deaths reviewed.[4] The most frequent suboptimal factors in this study were maternal smoking and failure to detect fetal growth restriction in the antenatal period. Assessment for the presence of potentially avoidable contributing factors or suboptimal care should also be recognized and documented in these multidisciplinary audit meetings and are the backbone of practice improvement through feedback of information and lessons learned.

In England and Norway, a national system of perinatal audit has been established. This has resulted in better collaboration around perinatal mortality, recommendations for guideline development, education and a higher quality of care.[5,6] In the Netherlands further national perinatal audit activities are being developed. In response to the Peristat results[2,3] the possible influence of substandard care was studied by a national feasibility study for audit of perinatal mortality: the National Perinatal Audit Study (LPAS). This study concluded that in 19% of perinatal mortality different substandard factors were possibly related to death and in 9% related to death and potentially avoidable.[7] Methods for initiation of national multidisciplinary perinatal audit were developed and the authors advised to organise local perinatal audit meetings. Subsequently, the study of Implementation of structural feedback by means of Perinatal Audit to Caregivers in cases of perinatal mortality in the northern region of the Netherlands (IMPACT) was set up. This projects aids in the initiation of structured multidisciplinary perinatal audit meetings for health care workers involved in perinatal mortality. The ministry of Health, Welfare and Sport (VWS) has supplied a grant until 2012 for national introduction of perinatal audit. From 2007 the National Institute for Public Health and the Environment (RIVM) further developed this national audit study. In June 2010 the PAN (Perinatal Audit Netherlands) foundation will further take over these activities. This foundation is an already active collaboration of the national professional organisations of midwives, gynaecologists, pathologists, paediatricians and general practioners working on perinatal audit. Their goal is to audit all Dutch cases of perinatal mortality. Our proposed multilayered approach for perinatal mortality including the Tulip classification is being used for these

national audit activities. National perinatal audit has three pillars: local, regional and national giving possibilities for investigation of national trends and areas of focus for training, amendment of guidelines and prevention. Essential in the process of implementation of audit is adequate and open communication regarding this issue. One of these aspects is coaching of professionals in the background of perinatal mortality, familiarity with use of classification systems and distribution of classification guidelines with agreements and case examples. This will enhance their motivation to participate in these meetings.

Consensus on classification of perinatal mortality

To accomplish comparison of European perinatal mortality rates we need to reach European consensus on classification of perinatal mortality. The European Board and College of Obstetrics and Gynaecology (EBCOG) should take the initiative to reach an agreement about this. Only then will we be able to identify why Dutch perinatal mortality figures are higher than the rest of Europe.

Consensus internationally on classification of perinatal mortality is the ultimate goal. Recently, the National Institute of Child Health and Human Development (NICHD) in the United States initiated an international stillbirth classification workshop. Participants came to a consensus regarding the pathophysiology of various conditions underlying stillbirth to improve the classification of causes of death.[8] These definitions of causes of death are the first step to achieve uniformity regarding allocation of causes. While there is evidence for some conditions to rise to the level of probable causes of death, there is still varying certainty in establishing other causes because of our incomplete understanding of the underlying pathophysiology. The Tulip classification is designed in such a way that future knowledge allows expansion or amendment of cause of death categories. Based on the occurrence of our reported placental pathologies in the ZOBAS study (studying valuable tests after intrauterine antepartum fetal death) we proposed amending the subgroups of placental causes of death in the Tulip classification. Essential in this process is always to go back to the initial, demonstrable pathophysiological entity initiating the chain of events that has irreversibly led to death when allocating a cause. There will be an ongoing process of amendments of cause of death categories as these are currently based on the existing knowledge we have on these entities.

Guideline for investigation of fetal death

As described in this thesis, to optimize investigation into cause of fetal death a 'golden standard' guideline is essential. On the base of our findings, we proposed an

intrauterine antepartum fetal death work-up guideline for diagnostic investigation in order to classify underlying cause of death. This proposal included a basic and an additional workup after fetal death based on our multidisciplinary evaluation of diagnostic procedures in determining cause of fetal death.

We concluded that autopsy, placental examination, cytogenetic analysis and testing for fetal-maternal haemorrhage are the base for diagnostic work-up for all fetal deaths. For further sequential testing, based on the results of these tests or clinical characteristics, serum for maternal virus serology and material for maternal, fetal and placental cultures or other microbiological diagnostic tests should be obtained and stored from all fetal deaths. The investigations for intrapartum deaths can be extracted from this protocol as the underlying causes are mostly the same. To implement these work-up findings nationally in daily patient care a national practice guideline will be extracted from our results in collaboration with the Dutch Society of Obstetrics and Gynaecology (NVOG). Earlier, fetal death workup protocols were based on expert opinion with controversies about what tests should be undertaken often resulting in hesitance of clinicians to adopt recommendations and a non-systematic approach to evaluation. The extent to which this suboptimal diagnostic approach is artificially inflating the number of unexplained stillbirths in the Netherlands is unknown. A side effect of implementation of our proposed evidence based work-up guideline could be an increase in deaths with a known cause, specially the group where important information was missing. Future studies should address this issue.

With adequate implementation of this fetal death work-up guideline through publicity, education and training, optimal uptake of these findings should be ensured. In Australia and New Zeeland, an educational program has been developed by the national Stillbirth Alliance Research Collaborative to assist with implementation of their stillbirth guidelines. The program is a small group, interactive, multi disciplinary skills training course, based on the SCORPIO model[9] designed to address the educational needs of all health professionals involved with childbirth and early newborn care.

Placental pathology

In this thesis, we concluded that more than half of the intrauterine antepartum fetal deaths were caused by placental pathology. Concerning this cause of death group there are two main issues that require attention and further research. Firstly, assessment of published work on placental pathologies and stillbirth is impeded by a general absence of standard definitions and nomenclature between studies. For example, for the discussed placental pathology villus immaturity there is not yet a general accepted definition. International consensus on definitions of different placental pathologies causing death needs to be further accomplished. Secondly,

the pathophysiology underlying both acute and chronic placental dysfunction is mostly unclear. With further investigation into the origin of these pathologies, occurrence and clinical manifestations, the cause of death categories will change. Based on differences in clinical manifestations we found evidence for separation of the cause placental hypoplasia and placental bed pathology. We subdivided the large group of fetal deaths caused by placental hypoplasia in relative and absolute hypoplasia. These two sub causes could be an expression of two different underlying pathophysiological entities. With more insight into this cause, placental hypoplasia could even become a clinical manifestation comparable to fetal growth restriction that was regarded as a cause of death earlier.

Cytogenetic analysis

Our results indicated that cytogenetic analysis for all intrauterine antepartum fetal deaths is valuable, preferably by invasive testing before labor as analysis of tissue postpartum had a high rate of failure. This implies that health care workers involved with fetal death should be able to perform amniocentesis or chorionic villus biopsy. If they do not have the expertise, couples should be referred to centres that perform invasive testing. Advances in molecular genetic technology will hopefully improve our ability to identify more genetic causes of stillbirth. Additional molecular cytogenetic analyses, such as fluorescent in situ hybridization, multiplex ligation-dependent probe amplification, comparative genomic hybridization and quantitative fluorescent polymerase chain reaction, have been advised if cells do not grow in culture. Although the numbers of these tests in our study were small, these techniques seem promising. The feasibility of these techniques in common practice needs further evaluation.

Thrombophilia

In our retrospective thrombophilia family cohort study, hereditary antithrombin and protein C deficiencies were associated with a high absolute risk of fetal death. An additional effect of cosegregation of other thrombophilic defects was not demonstrated. We concluded that this may be due to the exclusion of women at highest risk of venous thromboembolism. The group of women we studied is a group with non-prevalent hereditary thrombophilic deficiencies. Women from these families have often been confronted with thrombophilia related problems in their families or by their own around the age of childbearing. Some women have therefore already been tested for thrombophilic deficiencies in family setting. We must keep in mind that this is a special non-prevalent group of women with thrombophilia related risks. Our results support those of others that advise thrombophilia testing in women with fetal death with a family history of hereditary thrombophilia or a personal history of venous thromboembolism, in whom testing could help prevent further maternal venous thromboembolism.[10] In contrast to standard care in many hospitals, the need for routine testing of thrombophilic defects after intrauterine antepartum

fetal death was not supported by the results of the ZOBAS study. We showed that plasma levels of acquired thrombophilic defects changed during pregnancy. More women with fetal death had decreased antithrombin and protein C and increased von Willebrand factor plasma levels than pregnant women with uncomplicated pregnancies. However, compared to plasma levels in the normal population we only observed more women with fetal death with increased von Willebrand factor. Prevalence of inherited thrombophilias was not higher in couples with fetal death than in the normal population. Our results of main cause of death groups did not confirm the hypothesis that the pathophysiology of fetal death associated with thrombophilia is thrombosis in the uteroplacental circulation. Neither inherited nor acquired maternal or paternal thrombophilic defects were associated with main causes of death, but of placental causes abruption and infarction were associated with acquired maternal defects.

The clinical implications of these findings are yet unclear. Testing for abnormal levels of antithrombin, protein C, total protein S or von Willebrand factor may yield valuable predictors for a subgroup at risk for fetal death. However, thrombophilia screening should only be performed in cases where a proper management can be offered. As long as there are no randomized controlled trials proving the benefits of anticoagulant therapy in cases with known thrombophilic factors in relation to pregnancy outcome, we must be reserved about implementing a potentially harmful intervention in pregnant women.

Valuable tests

From our studies, we concluded that pathological examination consisting of autopsy and placental examination; and chromosomal analysis were valuable in the determination of cause of intrauterine antepartum fetal death. Parents should be informed about the reasons for and value of autopsy, placental examination and chromosomal analysis and their procedures. Health care workers involved in perinatal mortality should adequately counsel parents on these issues with respect to their background, religious and cultural aspects and wishes.[11] Ideally, parents should also be given written information explaining these aspects. In order to better equip health care workers in confronting bereaved parents with these emotional matters more education is needed for professionals on this issue. The use of standard pathology guidelines ensures that informative gross description and diagnostic histologic features are not overlooked. It assures the reader of the report that all features and aspects of the fetus and placenta have been critically examined. Whereas if the report only states abnormal findings a reader may be left wondering whether such critical histologic examination has been performed.[12] At the start of the ZOBAS study there were no national pathological guidelines for autopsy and placental examination. In collaboration with the Dutch Working

Party Paediatric Pathology (WKPLL) a pathological study protocol was prepared to accomplish more uniformity in the ZOBAS study. A national autopsy and placental examination guideline is now being extracted from this study protocol by the WKPLL for use for national perinatal audit. Hopefully, this guideline will receive a Dutch Institute for Healthcare Improvement (CBO) status in the near future. Such a guideline deserves the same implementation strategies under pathologists as the fetal death diagnostic work-up protocol for gynaecologists.

Performance of adequate autopsy and placental examination requires expertise in this field. It is fundamental to provide the pathologist with essential clinical information in guiding appropriate investigation into causes. Clinicians should request autopsies from the service providing the highest quality. Therefore, ideally a perinatal/paediatric pathologist should perform or supervise all perinatal post-mortems and placental examinations. If needed transport to a centre with appropriate expertise should be arranged to ensure that all examinations are of sufficient quality. Elsewhere this is already standard patient care.[13] A plain language pathology report for parents could give them more insight into findings and the value of pathological examination. Such a plain language report is already used for Dutch genetic counselling.

Developing countries

Ideally the complete proposed fetal death diagnostic workup guideline should be performed. However, this will not be feasible when resources are limited. While in many developing countries the ability to undertake the most basic diagnostic tests is extremely limited or even impossible, a systematic approach to collection and review of basic information from clinical history and examination of mother and baby should be achievable. Such data collection would constitute a major step forward in addressing stillbirth prevention on a global scale. When considering what investigations should be included in local stillbirth work-up in developing countries, it makes sense as we discussed in our proposal for a work-up guideline to focus on conditions suggested by the clinical history. In addition, there should be a focus on those disorders with meaningful recurrence risks. Population attributable risk (PAR) allows us to consider conditions that carry the largest burden in certain populations aiding in focussing and rationalising the approach to investigation.[14] Conditions with higher prevalence and higher risk have higher PAR. Malaria and syphilis for example have high stillbirth PAR's in developing countries. We proposed a basic and an additional workup after fetal death in our guideline. In collaboration with others, concerning diagnostic work-up internationally a four-tiered approach was presented with two main levels based on resource availability, each with additional testing dependent on the scenario. Level 1: Basic investigation. This level should be achievable in all settings regardless of the economic setting. Level

1S: Scenario specific investigation. These investigations may assist in identifying important contributing factors in specific situations where access to laboratory services and expertise is limited. Level 2: Optimal investigation for all stillbirths. In addition to Level 1, this approach is considered optimal and should be undertaken in all developed country settings. Level 2S: Optimal – scenario specific investigation. Additional testing based on clinical scenario which should be undertaken in all developed country settings.[14]

Psychological effects of fetal death

In addition to investigating the medical aspects of fetal death, it is important to consider the psychological effects on the family.[15] Parent support should include emotional support. Referral to a bereavement counsellor, religious leader, peer support group, or medical social worker is advisable for management of grief and depression. In the University Medical Centre Groningen the importance of psychosocial aftercare following perinatal deaths through a multidisciplinary approach coordinated by medical social workers has been studied. It was concluded that the aftercare group counselling sessions met a strong patient need.[16] Psychosocial problems in bereaved parents after perinatal loss need be further defined.

Prevention

This thesis gives more insight into occurrences of causes of intrauterine antepartum fetal death. With more insight into these causes, we can subsequently aim to bring down fetal death rates. This will also contribute to a decrease in perinatal morbidity. Bringing down fetal death rates can be achieved on different levels such as improving quality of care, studying risk factors and development of fetal testing during pregnancy for women at risk for fetal death.

Since the Peristat publications[2,3] different measures have been taken in the Netherlands in order to improve the quality of perinatal care. The most important are the preparations for the national perinatal audit, better prenatal screening and the introduction of preconception care.[17] Women at risk for adverse pregnancy outcome should be identified before pregnancy. The health care giver should perform a risk assessment for each individual patient and give realistic estimates of anticipated obstetric outcomes.

Risk factors

In developed countries, the most prevalent risk factors for stillbirth are obesity, socioeconomic factors, and advanced maternal age.[18] The prevalence of maternal

obesity is increasing steadily worldwide. Recent studies in the Netherlands indicated that women in the age group 20-39 years had the highest body-mass index (BMI) increase over the last years compared to other age groups.[19] In the ZOBAS study prevalence of mothers with a BMI > 30.0 was more than twice as high as in the age and sex matched general Dutch population. During pregnancy, these women had more hypertension and diabetes-related diseases compared to women in the other BMI groups and more intrauterine antepartum fetal deaths due to placental causes of death, namely placenta-bed pathology. For these obese women preconception advice, risk analysis and accurate identification of pregnancy complications is needed.[20] The fact that women with a previous stillbirth are at increased risk of stillbirth in future pregnancies is well known.[21] We studied women with previous fetal death and evaluated pregnancy outcome. There seems to be an association between previous fetal death and subsequent unfavourable fetal outcome related to the cause of death, especially in early gestation.[22] In terms of reducing potentially preventable stillbirths, the Confidential Inquiry into Stillbirths and Infant Death (CISID) of Northern Ireland found that the failure to adequately diagnose and manage fetal growth restriction was the most common error, followed by failure to recognize additional maternal medical risk factors.[23] It is also known that pregnancies affected by decreased fetal movements are at an increased risk of fetal growth restriction and fetal death.[24,25] Tveit et al. recently found, that combining improved guidelines for management of decreased fetal movements to health professionals and uniform information on fetal activity to expecting women, improved the quality of care and was associated with a reduction of stillbirth rates.[26] With more insight into the association between stillbirth risk factors and different causes of fetal death, better strategies to prevent death can be developed.

Fetal testing during pregnancy

We demonstrated that diverse placental pathologies are the main causes of intrauterine antepartum fetal death and that they vary in their clinical manifestations and depend on gestational age. Clinically useful gauges for detecting progressive placental failure during pregnancy need to be developed. Future research into fetal death will focus on understanding the pathophysiology of impaired placentation to establish tests for assessing risk of fetal death, and assessment of interventions to prevent fetal death in women who test positive. Up to now, studied fetal tests include circulating concentrations of placentally derived proteins in the mother's blood PAPP-A,[27,28] and α-fetoprotein (AFP),[29] doppler flowvelocimetry of the uterine and umbilical arteries,[30,31] and ultrasonic assessment of the appearance of the placenta.[32,33] Combining the results of these studies, with our demonstrated differences in gestational age occurrence of placental cause of death subgroups

can give more insight into possible timing of testing. We concluded that placental bed pathology had the highest occurrence of early intrauterine fetal death between 24 and 32 weeks and a strong decline after 32 weeks. In contrast, contribution of developmental placental pathology increased after 32 weeks of gestation. In doppler flow velocimetry studies of the uterine arteries a high resistance pattern of flow at the end of the second trimester of pregnancy was observed and associated with an increased risk of growth restriction and stillbirth.[31] Research on other fetal tests is still premature and has limited clinical use. Meta-analyses of methods of fetal monitoring do not suggest any methods of fetal assessment that reduce the risk of stillbirth when used in an unselected population. Some trials seem to show possible beneficial effects, such as assessment of placental maturity in the third trimester[32] but this has not been confirmed (or refuted) by any further trials.

Given all of the potential factors that influence the risk of fetal death, it would be helpful to have an interactive model that would estimate the risk for an individual pregnancy. This model could then be used to decrease the risk of fetal death by monitoring of pregnancy and informing decisions about the timing of delivery to prevent fetal death. However, delivery of the fetus incurs the risk of maternal or neonatal morbidity or mortality. The current management of risk conditions such as diabetes mellitus requiring insulin, hypertension-related disease, and fetal growth restriction already includes various schedules for testing during pregnancy. This proactive, comprehensive approach has led to the reduction of the stillbirth risk, albeit at the cost of an increased risk of iatrogenic preterm birth.[18]

An alternative approach to assessment of fetal wellbeing is to schedule delivery of women considered at high risk of stillbirth irrespective of the results of fetal tests. Villus immaturity, one of the placental causes of fetal death we studied, characterised by a reduced number of syncytiovascular membranes in the tertiary villi can not yet be clinically diagnosed.[34,35] In our study we observed an association with gestational diabetes. Up to now, no other markers seem to correlate with this histopathological finding. Stallmach et al. proposed that these fetuses can be rescued by birth as their placenta is required to function for only a few more days and suggested birth at 37 completed weeks of gestation.[34,35] There are however no data from randomised controlled trials that directly support timing of delivery because of the difficulty in doing adequately powered studies for these women. Further studies are needed to design interventions for pregnant women at risk of fetal death that will reduce the incidence of fetal death and lead to the birth of a healthy newborn.

References

1. Galan-Roosen T. Perinatal Mortality: registration, classification of causes of death and risk factors. Katholieke Universiteit Nijmegen, 2002.
2. Buitendijk S, Zeitlin J, Cuttini M, Langhoff-Roos J, Bottu J. Indicators of fetal and infant health outcomes. Eur J Obstet Gynecol Reprod Biol. 2003;111 Suppl 1:S66-S77.
3. Mohangoo AD, Buitendijk SE, Hukkelhoven CW et al. Higher perinatal mortality in The Netherlands than in other European countries: the Peristat-II study. Ned Tijdschr Geneeskd. 2008;152:2718-2727.
4. Richardus JH, Graafmans WC, Verloove-Vanhorick SP, Mackenbach JP. Differences in perinatal mortality and suboptimal care between 10 European regions: results of an international audit. BJOG. 2003;110:97-105.
5. Acolet D, Springett A, Golightly S. Confidential Enquiry into Maternal and Child Health (CEMACH): Perinatal Mortality 2006: England, Wales and Northern Ireland. 1-102. 2009. London. 1-4-2008.
6. Bergsjo P, Bakketeig LS, Langhoff-Roos J. The development of perinatal audit: 20 years' experience. Acta Obstet Gynecol Scand. 2003;82:780-788.
7. Eskes M and Van Diem MTh. National Perinatal Audit Study. 231. 2005. Diemen, College voor Zorgverzekeringen.
8. Reddy UM, Goldenberg R, Silver RM 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.
9. Hill DA. SCORPIO: a system of medical teaching. Med Teach. 1992;14:37-41.
10. Middeldorp S. Thrombophilia and pregnancy complications: cause or association? J Thromb Haemost. 2007;5 Suppl 1:276-282.
11. Gordijn SJ, Erwich JJ, Khong TY. The perinatal autopsy: pertinent issues in multicultural Western Europe. Eur J Obstet Gynecol Reprod Biol. 2007;132:3-7.
12. Khong TY, Gordijn SJ. Quality of placental pathology reports. Pediatr Dev Pathol. 2003;6:54-58.
13. The Perinatal Mortality Special Interest Group of the Perinatal Society of Australia and New Zealand. Clinical Practice Guideline for Perinatal Mortality Audit. 2004.
14. Flenady V, Silver RM, Incerpi M et al. Essential diagnostic work-up of stillbirths. In: Facchinetti F, Dekker GA, Saade G, eds. Stillbirth: understanding and management. London: Informa Healthcare UK Ltd; 2009:1-28.
15. Bartellas E, Van AJ. Bereavement support for women and their families after stillbirth. J Obstet Gynaecol Can. 2003;25:131-138.
16. de Groot-Noordenbos M, van der Berg PP, Wieman L, Erwich JJ. The importance of psychosocial aftercare following perinatal deaths: The UMCG multidisciplinary approach and the significance of medical social work: a model for support groups. University Medical Centre Groningen. Abstract 2008.
17. Merkus JM. Obstetric care in The Netherlands under assessment again. Ned Tijdschr Geneeskd. 2008;152:2707-2708.
18. Fretts RC. Etiology and prevention of stillbirth. Am J Obstet Gynecol. 2005;193:1923-1935.

19. Gast GC, Frenken FJ, van Leest LA, Wendel-Vos GC, Bemelmans WJ. Intra-national variation in trends in overweight and leisure time physical activities in The Netherlands since 1980: stratification according to sex, age and urbanisation degree. Int J Obes (Lond). 2007;31:515-520.

20. Korteweg FJ, Erwich JJHM, Timmer A, van der Meer J, Ravisé JM, Holm JP. Meer kans op intra-uteriene vruchtdood bij extreem overgewicht. NTOG. 2007;33-37.

21. Samueloff A, Xenakis EM, Berkus MD, Huff RW, Langer O. Recurrent stillbirth. Significance and characteristics. J Reprod Med. 1993;38:883-886.

22. Nijkamp JW, Korteweg FJ, Holm, JP, Timmer A, Erwich JJ, van Pampus MG. Subsequent pregnancy outcome after previous fetal death. Submitted 2009.

23. Kady M, Gardosi J. Perinatal mortality and fetal growth restriction. Best Pract Res Clin Obstet Gynaecol. 2004;18:397-410.

24. Olesen AG, Svare JA. Decreased fetal movements: background, assessment, and clinical management. Acta Obstet Gynecol Scand. 2004;83:818-826.

25. Sinha D, Sharma A, Nallaswamy V, Jayagopal N, Bhatti N. Obstetric outcome in women complaining of reduced fetal movements. J Obstet Gynaecol. 2007;27:41-43.

26. Tveit JV, Saastad E, Stray-Pedersen B et al. Reduction of late stillbirth with the introduction of fetal movement information and guidelines - a clinical quality improvement. BMC Pregnancy Childbirth. 2009;9:32.

27. Dugoff L, Hobbins JC, Malone FD et al. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). Am J Obstet Gynecol. 2004;191:1446-1451.

28. Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy levels of pregnancy-associated plasma protein a and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. J Clin Endocrinol Metab. 2002;87:1762-1767.

29. Waller DK, Lustig LS, Smith AH, Hook EB. Alpha-fetoprotein: a biomarker for pregnancy outcome. Epidemiology. 1993;4:471-476.

30. Baschat AA, Gembruch U, Weiner CP, Harman CR. Qualitative venous Doppler waveform analysis improves prediction of critical perinatal outcomes in premature growth-restricted fetuses. Ultrasound Obstet Gynecol. 2003;22:240-245.

31. Lees C, Parra M, Missfelder-Lobos H, Morgans A, Fletcher O, Nicolaides KH. Individualized risk assessment for adverse pregnancy outcome by uterine artery Doppler at 23 weeks. Obstet Gynecol. 2001;98:369-373.

32. Proud J, Grant AM. Third trimester placental grading by ultrasonography as a test of fetal wellbeing. Br Med J (Clin Res Ed). 1987;294:1641-1644.

33. Viero S, Chaddha V, Alkazaleh F et al. Prognostic value of placental ultrasound in pregnancies complicated by absent end-diastolic flow velocity in the umbilical arteries. Placenta. 2004;25:735-741.

34. Stallmach T, Hebisch G, Meier K, Dudenhausen JW, Vogel M. Rescue by birth: defective placental maturation and late fetal mortality. Obstet Gynecol. 2001;97:505-509.

35. Stallmach T, Hebisch G. Placental pathology: its impact on explaining prenatal and perinatal death. Virchows Arch. 2004;445:9-16.

Summary

Fetal death or stillbirth is a major obstetrical complication and a devastating experience for parents. Health care workers are responsible for investigating the cause of death. Unfortunately, the cause remains unexplained in up to two-thirds of fetal deaths. This is partly influenced by lack of consensus on classification of cause of fetal death and diagnostic investigations into causes.
Different aspects of classification systems for cause of perinatal mortality were investigated. In this thesis, the main focus is on optimal investigation of cause of intrauterine antepartum fetal death. Therefore, we studied the value of different diagnostics in allocating the underlying cause. The aim of this thesis was to propose an evidence based guideline for diagnostic work-up after fetal death to determine the cause of death.

Part I: Classification of perinatal mortality

In Part I we focused on diverse aspects of different classification systems for perinatal mortality. In **Chapter 1,** the pathophysiological Tulip classification system for underlying cause and mechanism of perinatal mortality is introduced based on clinical and pathological findings. This classification, consisting of groups of causes and mechanism of death was drawn up by a multidisciplinary panel through the causal analysis of events related to 411 studied perinatal mortalities. The underlying cause of death was defined as the initial demonstrable pathophysiological entity initiating the chain of events that has irreversibly led to death. The classification system consists of six main causes: 1. congenital anomaly, 2. placenta, 3. prematurity, 4. infection, 5. other and 6. unknown. Mechanisms of death defined as the organ failure incompatible with life and the origins of the mechanism were also drawn up. In addition, contributing factors were defined as other known factors on the causal pathway to death. Clear definitions and guidelines for case allocation with case examples were developed. The largest cause of death group was congenital anomalies (35%). Cause of death was unknown in only 11%. Inter-rater agreement between five panel members expressed by kappa score was 0.81 for main cause of death (0.86 after excluding guideline misinterpretations), 0.67 for subclassification of cause of death and 0.72 for mechanism of death. The best agreement level for cause of death was observed for “congenital anomaly” and the lowest for “other”. Classifying perinatal mortality to compare performance over time and between centres is useful and necessary for different purposes. The Tulip classification seems consistent and allows unambiguous classification of a single underlying cause and mechanism of perinatal mortality. It is easily applicable in a team of clinicians when guidelines are followed.

Differences between perinatal mortality classification systems could have consequences for the validity of vital statistics, for targeting preventive strategies and also for counselling parents on recurrence risks. In **Chapter 2,** this was illustrated by comparing the use of the Tulip classification with other currently used international classification systems for cause of fetal death. We selected the extended Wigglesworth classification, modified Aberdeen, ReCoDe and the classifications by Hey, Hovatta, Galan-Roosen and Morrison and Olsen. Our multidisciplinary panel classified cause of 485 intrauterine antepartum fetal deaths according to the different systems after individual investigation of structured patient information. Distribution of cases into cause of death groups for the different systems varied. The proportion of cases in the placental groups varied from 0% (no placental category provided in those systems) to 65% in the Tulip classification, the largest cause of death group. In some systems, cases with an unexplained cause of death comprised the largest group such as in the extended Wigglesworth (86%), while in other systems no deaths were classified as unexplained because death groups consisted of clinical manifestations. The most frequent contributing factor was growth restriction. Systems that lack a placental category and systems that allocate most cases to the "unknown" categories or to categories that comprise only clinical manifestations are not discriminatory for the underlying cause of death. In the Tulip classification, mother, fetus and placenta are addressed together and the system has a clear defined sub classification of the placenta group.

There is not a universally accepted classification system for perinatal mortality. All systems have their own strengths and weaknesses. In **Chapter 3,** we compared all known international classification systems regarding their definition of the perinatal period; level of complexity; inclusion of maternal, fetal and/or placental factors; and whether they focus on a clinical or pathological viewpoint. We allocated these classification systems to one of three categories: 'when', 'what' or 'why'. This was dependent on whether the allocation of the individual cases of perinatal mortality was based on the moment of death ('when'), the clinical conditions associated with death: fetal, maternal or placental ('what'), or the underlying cause of death: the event that initiated the chain of events that eventually resulted in death ('why'). This led to proposal of a systematic multilayered approach for the analysis of perinatal mortality by using combinations of existing classification systems. Cross tables of the different systems can give insight into the relation between 'when', 'what' and 'why'. If causes and conditions are mixed within a system, overlap in allocation is possible, information is then lost and comparison is unreliable. When cause and condition are used separately, they may add to each other. This approach is not only useful for in depth analysis of perinatal mortality in the developed world

but also for analysis of perinatal mortality in developing countries, with limited resources.

Part II: Value of diagnostic tests after intrauterine antepartum fetal death

In Part II of this thesis value of different diagnostic tests for allocation of causes of intrauterine antepartum deaths according to the Tulip classification by a multidisciplinary panel was evaluated. The results of the prospective ZOBAS study of couples and their singleton intrauterine fetal death after 20 weeks of gestation before the onset of labor are presented in the following chapters. **Chapter 4** describes different placental pathologies causing fetal death in 750 cases. Placental pathology was the most dominant cause of death group in 65%, becoming relatively more important at higher gestational age. Placental bed pathology was observed most of all; in 34% of all fetal deaths, with the highest occurrence between 24 and 32 weeks and a strong decline after 32 weeks. In contrast, contribution of developmental placental pathology (18%) increased after 32 weeks of gestation ($p<0.001$), as did umbilical cord complications (5%) and combined placental pathology (6%). In the placental bed pathology group more hypertension-related disease and small for gestational age fetuses were observed compared to other cause of death groups. However, more than one third of cases with placental bed pathology did not present with these clinical manifestations. Diabetes-related disease was particularly observed in the group of deaths due to placental hyopoplasia. We concluded that different placental pathologies were associated to different gestational age periods, and that clinical manifestations varied during pregnancy.

One of the placental causes of death we studied is the relatively unknown villus immaturity described in **Chapter 5,** causing unexpected antepartum fetal death after 36 weeks of gestation. In our complete ZOBAS cohort of 1025 intrauterine antepartum fetal deaths, 352 were beyond 36 weeks of gestation. These fetal deaths were divided into three cause of death groups: villus immaturity, other placental pathology and non-placental pathology. A placental cause of death was identified in almost 80%. The overall prevalence of villus immaturity was 23%, either solitary or in combination with other placental pathology. The prevalence of gestational diabetes in the villus immaturity group was 2.5 fold-higher than in the group caused by other placental pathology (14% versus 6%, $p=0.03$) and 10 fold- higher than in the group caused by non-placental pathology (14% versus 1%, $p=0.005$). Villus immaturity was also associated with placental

hypoplasia (developmental pathology) in comparison to the group of deaths with a non-placental cause. No associations were found for oligohydramnios although this occurred almost twice as often in the group with villus immaturity (23%) than in the group with non-placental causes (13%, p=0.14); hyper coiling of the umbilical cord; pre-existent diabetes mellitus; hypertensive-related disease; intoxications (smoking, alcohol, drugs); or fetal characteristics.

Chapter 6 describes chromosomal abnormalities causing fetal death. The aim of the study was to estimate success rates for cytogenetic analysis in different types of tissue after evaluation of 750 intrauterine antepartum fetal deaths. In addition, we studied selection criteria for and value of cytogenetic testing in determining cause of death. We observed chromosomal abnormalities in 13% of deaths. Cytogenetic success rates were significantly higher for invasive testing (85%) than for postpartum tissue analysis (28%, p<0.001). Success rates of tissues taken postpartum varied between 32% for umbilical cord and 0% for pericardium. A small for gestational age fetus or advanced maternal age (above 35 years) were not associated with more chromosomal abnormalities. Cytogenetic analysis was successful in 35% of severely macerated fetuses. There were more abnormal chromosomes (38%) in fetal deaths with morphologic abnormalities than in those without (5%, p<0.001). However, the posterior probability of a chromosomal abnormality in the absence of morphologic abnormalities was still 5%. Cytogenetic analysis was valuable in determining the cause in 19% of fetal deaths. The results of this study led to recommendations for a fetal death cytogenetic flowchart. We suggest counselling parents on different aspects of cytogenetic analysis and discussing their consequences, and performing non-selective invasive testing after fetal death and before labor for all intrauterine antepartum fetal deaths.

In **Chapter 7,** a retrospective family cohort study (Descartes study) is presented with absolute risks of fetal loss for women with hereditary deficiencies of either antithrombin, protein C and S compared to their non-deficient female relatives. Evaluable were 317 women, who had 987 pregnancies (582 in 185 deficient women). Total fetal loss rates were 47% (antithrombin deficient), 45% (protein C deficient), 21% (protein S type I deficient) and 30% (protein S type III deficient), compared to 32%, 28%, 29% and 27% in non-deficient women, respectively. Adjusted for clustering of pregnancies in women, and compared to all non-deficient women, relative risks for fetal death were 2.3 (95% CI, 0.9-6.1) in antithrombin deficient women, 2.1 (95% CI 0.9-4.7) in protein C deficient women, 0.7 (95% CI 0.2-1.8) in protein S type I deficient women and 1.1 (95% CI 0.6-2.0) in protein S type III deficient women. Early fetal death rates showed no statistically significant differences between deficient and non-deficient women. Differences

were mainly due to higher late fetal death rates in antithrombin (adjusted relative risk 11.3; 95% CI, 3.0-42.0) and protein C deficient women (adjusted relative risk 4.7; 95% CI, 1.3-17.4). An additional effect of cosegregation of other trombophilic defects: factor V Leiden and/or prothrombin G20210A was not demonstrated in both groups. Beforehand however, we excluded pregnancies after prior venous thromboembolism because thromboprophylaxis may have influenced the outcome, which could explain our cosegregation findings. These women probably have a high risk of fetal loss.

In **Chapter 8,** we describe the prevalence of maternal thrombophilic defects, either inherited or acquired during pregnancy and paternal thrombophilic defects, tested at induction of labor in the ZOBAS cohort (n=750) compared to prevalence in the normal population. Prevalence of inherited thrombophilias was no higher in couples with fetal death than in the normal population. More women with fetal death had decreased antithrombin (16.8%) and protein C (4.0%) and increased von Willebrand factor (15.5%) plasma levels compared to healthy pregnant women (2.5%). However, compared to normal ranges in the non-pregnant population, only more women with fetal death were observed with increased von Willebrand factor (12.4%). More fathers with fetal death had decreased free protein S and elevated von Willebrand factor than healthy men. When comparing main causes of death, thrombophilia was not associated with a placental cause of death, presumed to express thrombosis in the uteroplacental circulation. After studying specific placental causes, abruption and infarction were associated with acquired maternal defects. In contrast with common clinical practice, our data provide no support for routine testing of inherited or acquired thrombophilic defects after fetal death, although acquired maternal defects may play a role in deaths caused by abruption or infarction.

Part III: Fetal death workup guideline

In the last part of this thesis we present recommendations for a basic and selective workup guideline for intrauterine antepartum fetal death. This workup is presented in **Chapter 9** by means of a flowchart for a diagnostic workup guideline based on the identification of valuable tests for determining the cause of death by a multidisciplinary evaluation of diagnostic procedures in the complete ZOBAS cohort of 1025 intrauterine antepartum fetal deaths. Beforehand an extensive non-selective diagnostic workup was performed for all deaths. Main causes of death were placental pathology (65.2%), congenital anomaly (4.8%), infection (1.8%) and other (5.0%) while in 23.2% cause remained unknown. The most

valuable tests for determination of cause of death were placental examination in 98%, autopsy in 73% and cytogenetic analysis in 29%. Fetal-maternal haemorrhage determined by a positive Kleihauer-Betke was observed in 12% of women. IgM antibodies against viruses and Toxoplasmosis were positive in 18%, but in only 1.8% placental examination and/or autopsy were able to support an intrauterine infection as cause of death. Testing for maternal diseases was regarded valuable if there was a suspect clinical history or suspect current pregnancy. Autopsy, placental examination and cytogenetic analysis are the base for diagnostic work-up for all fetal deaths. We recommend further individualised sequential testing on the base of these results or specific clinical characteristics to avoid unnecessary investigations and positive test results that do not identify the cause of fetal death.

Samenvatting

Foetale sterfte of doodgeboorte is een zeer ernstige obstetrische complicatie en is voor ouders een dramatische gebeurtenis. Hulpverleners zijn verantwoordelijk voor het achterhalen van de oorzaak van de sterfte. Bij tot wel tweederde van de sterfgevallen blijft die doodsoorzaak helaas onbekend. Gebrek aan consensus over classificatie van de oorzaak van foetale sterfte en onduidelijkheid over de te verrichten diagnostische onderzoeken om de doodsoorzaak vast te stellen zijn hier medeverantwoordelijk voor.
Verschillende aspecten aangaande classificatiesystemen voor perinatale sterfte worden beschreven. Centraal in dit proefschrift staat de vraag welke onderzoeken zinnig zijn en zouden moeten worden verricht om de oorzaak van een intra-uteriene antepartum vruchtdood (IUVD) te kunnen vaststellen. De waarde van verschillende individuele diagnostische onderzoeken na IUVD werd bestudeerd. Doel van dit proefschrift was om een evidence-based richtlijn voor diagnostisch onderzoek op te stellen om de oorzaak van een IUVD te kunnen vaststellen.

Deel I: Classificatie van perinatale sterfte

In Deel I van dit proefschrift worden verschillende aspecten van internationaal gebruikte classificatiesystemen voor perinatale sterfte bestudeerd. In **Hoofdstuk 1** wordt het pathosfysiologische Tulip classificatiesysteem voor onderliggende doodsoorzaak en mechanisme van perinatale sterfte, gebaseerd op klinische en pathologische bevindingen geïntroduceerd. Deze classificatie, die bestaat uit groepen doodsoorzaken en mechanismen van overlijden werd opgesteld door een multidisciplinair panel na analyse van gebeurtenissen gerelateerd aan 411 perinatale sterftes. De onderliggende doodsoorzaak werd gedefinieerd als de initiële aantoonbare pathofysiologische entiteit die de keten van gebeurtenissen met als gevolg de dood onomkeerbaar in gang heeft gezet. Dit classificatiesysteem bestaat uit zes hoofdgroepen doodsoorzaken: 1. aangeboren afwijkingen, 2. placenta, 3. prematuriteit, 4. infectie, 5. anders en 6. onbekend. Het mechanisme van overlijden, gedefinieerd als het orgaanfalen dat onverenigbaar is met leven en de oorsprong van dit mechanisme werden vastgesteld. Tevens werden bijdragende factoren gedefinieerd als andere bekende factoren die van invloed waren op het causale pad naar de dood. Heldere definities en richtlijnen voor toewijzing van casus met voorbeelden werden ontwikkeld. De grootste groep van doodsoorzaken bleek aangeboren afwijkingen (35%). De oorzaak van overlijden was in slechts 11% van de gevallen onbekend. Interbeoordelaar overeenstemming tussen vijf panelleden, weergegeven door een kappascore, was 0.81 voor hoofdgroepen doodsoorzaken (0.86 na exclusie van misclassificatie wegens misinterpretaties van de richtlijn), 0.67 voor de subclassificatie van de doodsoorzaak en 0.72 voor

het mechanisme van overlijden. De beste overeenstemming werd gevonden voor de doodsoorzaak “aangeboren afwijkingen” en de slechtste voor “anders”. Het classificeren van perinatale sterfte is nuttig voor het vergelijken van kwaliteit van zorg over een tijdsperiode en tussen ziekenhuizen en is tevens noodzakelijk voor andere doeleinden. De Tulip classificatie lijkt consistent en eenduidig voor classificatie van één onderliggende oorzaak en mechanisme van overlijden. Het is gemakkelijk toepasbaar in de kliniek als men de richtlijnen aanhoudt.

Verschillen tussen classificatiesystemen voor perinatale sterfte hebben consequenties voor de validiteit van statistieken, het bepalen van preventieve strategieën en het counselen van ouders aangaande herhalingsrisico’s. In **Hoofdstuk 2** wordt dit geïllustreerd door de Tulip classificatie te vergelijken met andere huidige gebruikte internationale classificatiesystemen voor oorzaak van doodgeboorte. Hiervoor werden de extended Wigglesworth classificatie, modified Aberdeen, ReCoDe en de classificatiesystemen opgesteld door Hey, Hovatta, Galan-Roosen en Morrison en Olsen geselecteerd. Na individuele bestudering van gestructureerde patiënteninformatie classificeerde het multidisciplinaire panel de oorzaak van 485 intra-uteriene antepartum sterftes in de verschillende systemen. De verdeling van de doodsoorzaken varieerden tussen de verschillende systemen. Het aandeel sterftes in de placentaire groepen varieerde van 0% (systemen zonder placentaire doodsoorzaak) tot 65% in de Tulip classificatie, waar het de grootste doodsoorzaakgroep was. In sommige systemen was een onbekende doodsoorzaak de grootste groep, zoals in de extended Wigglesworth (86%) terwijl in andere systemen geen enkele casus werd geclassificeerd als onbekend omdat de groepen doodsoorzaken werden weergegeven door klinische manifestaties. De meest voorkomende bijdragende factor was intra-uteriene groeirestrictie. Classificatiesystemen die geen placentacategorie hebben, die alleen bestaan uit klinische manifestaties of waarbij de meeste sterftes in de categorie onbekende doodsoorzaak worden geclassificeerd geven geen onderscheid voor de onderliggende doodsoorzaak. In de Tulip classificatie worden moeder, foetus en placenta als één geheel beschouwd waarbij er een heldere gedefinieerde subclassificatie aanwezig is in de groep placentaire doodsoorzaken.

Er bestaat geen universeel geaccepteerd en gebruikt classificatiesysteem voor perinatale sterfte. Alle systemen hebben hun eigen sterke en zwakke punten. In **Hoofdstuk 3** zijn alle bekende internationaal gebruikte classificatiesystemen vergeleken voor wat betreft de definitie van de perinatale periode; het niveau van complexiteit; de inclusie van maternale, foetale en/of placentaire factoren; en of het systeem was opgesteld vanuit een klinisch of pathologisch uitgangspunt. Deze verschillende classificatiesystemen werden vervolgens ingedeeld in één van de

drie categorieën: 'wanneer', 'wat' of 'waarom'. Allocatie van individuele casus van perinatale sterfte was gebaseerd op het moment van overlijden ('wanneer'), op klinische manifestaties geassocieerd met het overlijden ('wat'), of op de onderliggende doodsoorzaak: de initiële aantoonbare pathofysiologische entiteit die het mechanisme naar de dood onomkeerbaar in gang heeft gezet ('waarom'). Dit resulteerde in een systematische gelaagde benadering van perinatale sterfte door gebruik van verschillende classificatiesystemen. Kruistabellen van de verschillende systemen geven inzicht in de relatie tussen 'wanneer', 'wat' en 'waarom'. Indien doodsoorzaken en klinische manifestaties binnen één systeem door elkaar worden gebruikt kan dit leiden tot overlap tussen categorieën, waarbij informatie verloren gaat en vergelijken onbetrouwbaar wordt. Als doodsoorzaken en klinische manifestaties apart van elkaar worden geclassificeerd vullen ze elkaar aan. Deze benadering is niet alleen bruikbaar voor diepteanalyse van perinatale sterfte in ontwikkelde landen maar ook voor analyse in ontwikkelingslanden met beperkte middelen.

Deel II: Waarde van diagnostische onderzoeken na intra-uteriene antepartum sterfte

In Deel II wordt de waarde van verschillende diagnostische onderzoeken voor het bepalen van de oorzaak van een intra-uteriene antepartum sterfte door een multidisciplinair panel met gebruikmaking van de Tulip classificatie geëvalueerd. In de volgende hoofdstukken worden de resultaten gepresenteerd van de prospectieve ZOBAS studie van paren met een intra-uteriene antepartum sterfte van een eenling na 20 weken zwangerschapsduur. In **Hoofdstuk 4** worden verschillende placentaire afwijkingen die foetale sterfte veroorzaakten in een cohort van 750 casus beschreven. Placentapathologie was in 65% de doodsoorzaak. Bij toename van de zwangerschapsduur liep dat percentage op. Placentabedpathologie (34%) werd vooral gezien tussen 24 en 32 weken zwangerschapsduur met een sterke daling na 32 weken. De bijdrage van placentaire ontwikkelingsstoornissen (18%) daarentegen nam toe na 32 weken ($p<0.001$), evenals navelstrengcomplicaties (5%) en gecombineerde placentaire pathologie. In de groep met placentabedpathologie werd vergeleken met de andere groepen meer hypertensiegerelateerde ziekten gezien en foetussen die te licht waren voor de zwangerschapsduur. Toch werden deze klinische manifestaties bij meer dan een derde van de casus met placentabedpathologie niet gezien. Diabetesgerelateerde ziekte werd voornamelijk waargenomen in de groep vruchtdoden veroorzaakt door placentahypoplasie. Foetale sterfte door verschillende placentaire afwijkingen bleek geassocieerd met verschillen in gestatieduur en klinische manifestaties.

Een van de placentaire doodsoorzaken, de relatief onbekende villusimmaturiteit die onverwachte antepartum sterfte na 36 weken zwangerschapsduur kan veroorzaken is beschreven in **Hoofdstuk 5**. In ons complete ZOBAS cohort van 1025 intra-uteriene antepartum sterftes traden 352 na een termijn van 36 weken op. Op basis van de doodsoorzaak werden deze casus opgedeeld in drie groepen: villusimmaturiteit, andere placentaire doodsoorzaken en niet-placentaire doodsoorzaken. In 80% van deze casus werd de dood veroorzaakt door placentapathologie. In de groep na 36 weken werd in 23% villusimmaturiteit vastgesteld, solitair of in combinatie met andere placentapathologie. Het voorkomen van zwangerschapdiabetes in de villusimmaturiteitgroep was 2.5 keer hoger dan in de groep waarbij de dood veroorzaakt werd door andere placentaire pathologie (14% versus 6%, $p=0.03$) en 10 keer hoger dan in de groep veroorzaakt door niet-placentaire pathologie (14% versus 1%, $p=0.005$). Villusimmaturiteit was eveneens geassocieerd met placentahypoplasie in tegenstelling tot de groep vruchtdoden met een niet-placentaire doodsoorzaak. Er werden geen associaties gevonden met oligohydramnion, hoewel dit wel twee keer zo vaak in de villusimmaturiteit groep (23%) als in de groep met een niet-placentaire doodsoorzaak (13%, $p=0.14$) voorkwam, hypercoiling van de navelstreng, pre-existente diabetes mellitus, hypertensiegerelateerde ziekte, intoxicaties (roken, alcohol, drugs) en foetale karakteristieken.

In **Hoofdstuk 6** worden chromosomale afwijkingen, die foetale sterfte kunnen veroorzaken, beschreven. Het doel van deze studie was om na evaluatie van 750 intra-uteriene antepartum sterftes percentages te bepalen voor succesvolle analyse van cytogenetisch onderzoek in verschillende weefseltypes. Tevens werden selectiecriteria voor en waarde van cytogenetisch onderzoek voor het vaststellen van de doodsoorzaak bestudeerd. In 13% was er sprake van een chromosomale afwijking. Succesvolle cytogenetische analyse was significant hoger voor invasieve testen antepartum (85%) dan voor postpartum weefselanalyse (28%, $p<0.001$). Het aanslaan van weefselkweken van postpartum verkregen materiaal varieerde van 32% voor navelstreng- tot 0% voor pericardweefsel. Een foetus met een te licht gewicht voor de zwangerschapsduur of een maternale leeftijd boven de 35 jaar waren niet geassocieerd met chromosomale afwijkingen. Cytogenetische analyse was succesvol bij 35% van de ernstig gemacereerde foetussen. In de groep vruchtdoden met morfologische afwijkingen waren er meer abnormale karyotyperingen dan in de groep zonder (5%, $p<0.001$). De achteraf kans op een chromosomale afwijking bij een foetus zonder morfologische afwijkingen was echter nog steeds 5%. Cytogenetisch onderzoek was in 19% waardevol voor het vaststellen van de oorzaak van de vruchtdood. De resultaten van deze studie hebben geleid tot een flowschema voor cytogenetische analyse

bij een intra-uteriene vruchtdood. Ouders moeten worden voorgelicht over de verschillende aspecten van cytogenetisch onderzoek en de consequenties hiervan. Bij iedere intra-uteriene antepartum sterfte bestaat een indicatie voor invasief cytogenetisch onderzoek vòòr de bevalling.

In **Hoofdstuk 7** wordt een retrospectieve familiecohortstudie (Descartes studie) gepresenteerd waarbij absolute foetale sterfterisico's van vrouwen met erfelijke deficiënties van antithrombine, proteïne C of S vergeleken worden met niet-deficiënte vrouwelijke familieleden. De 317 evalueerbare vrouwen hadden 987 zwangerschappen (582 in 185 deficiënte vrouwen). Foetale sterfte trad op bij 47% (antithrombine deficiënt), 45% (proteïne C deficiënt), 21% (proteïne S type I deficiënt) en 30% (proteïne S type III deficiënt), vergeleken met respectievelijk 32%, 28%, 29% and 27% van de niet-deficiënte vrouwen. Gecorrigeerd voor clustering van zwangerschappen van vrouwen en vergeleken met alle niet-deficiënte vrouwen, waren de relatieve risico's voor foetale sterfte respectievelijk 2.3 (95% CI 0.9-6.1), 2.1 (95% CI 0.9-4.7), 0.7 (95% CI 0.2-1.8) en 1.1 (95% CI 0.6-2.0). Vroege foetale sterfte liet geen significant verschil zien tussen deficiënte en niet-deficiënte vrouwen. De gevonden verschillen waren voornamelijk het gevolg van een hogere frequentie van late foetale sterfte bij antithrombine (gecorrigeerde relatief risico 11.3; 95% CI 3.0-42.0) en proteïne C deficiënte vrouwen (gecorrigeerde relatief risico 4.7; 95% CI 1.3-17.4). De aanwezigheid van cosegregatie van factor V Leiden en/of prothrombin G20210A had geen effect op het risico van foetale sterfte in beide groepen. Zwangerschappen die optraden na eerdere veneuze trombose werden echter op voorhand geëxcludeerd omdat gebruik van tromboseprofylaxe de uitkomst van deze zwangerschappen zou kunnen hebben beïnvloed. Dit zou onze cosegregatie uitkomsten kunnen verklaren. Deze vrouwen hadden mogelijk een hoog risico op foetale sterfte.

In **Hoofdstuk 8** wordt de prevalentie van zowel erfelijke als verworven maternale en paternale thrombofiele afwijkingen getest tijdens inductie van de baring in het ZOBAS cohort (n=750) vergeleken met de prevalentie ervan in de normale populatie. Het voorkomen van erfelijke thrombofilie was in paren met een foetale sterfte niet hoger dan in de normale populatie. Meer vrouwen met een vruchtdood hadden verlaagde antithrombine (16.8%) en proteïne C (4.0%) en verhoogde von Willebrandfactor (15.5%) plasmaspiegels vergeleken met gezonde zwangeren (2.5%). Wanneer deze plasmaspiegels echter vergeleken werden met normaalwaardes in de niet-zwangere populatie waren er alleen meer vrouwen met een vruchtdood met een verhoogde von Willebrand factor (12.4%). Meer vaders met een intra-uteriene vruchtdood hadden een verlaagde vrij proteïne S en verhoogde von Willebrand factor vergeleken met gezonde mannen. Na vergelijk

van de verschillende groepen van doodsoorzaken waren thrombofiele afwijkingen niet geassocieerd met een placentaire doodsoorzaak, mogelijk een uiting van trombose in de uteroplacentaire circulatie. Na bestudering van de placentaire subgroepen waren abruptio en infarcten wel geassocieerd met verworven maternale thrombofiele afwijkingen. In tegenstelling tot de huidige klinische praktijk, bieden onze resultaten geen onderbouwing voor het routinematig testen van erfelijke of verworven thrombofiele afwijkingen na intra-uteriene vruchtdood. Verworven maternale thrombofiele afwijkingen spelen mogelijk wel een rol bij abruptio of infarcten.

Deel III: Diagnostische richtlijn bij foetale sterfte

In **Hoofdstuk 9** van dit proefschrift presenteren we aanbevelingen voor een richtlijn voor basaal en selectief diagnostisch onderzoek na intra-uteriene antepartum vruchtdood. Deze richtlijn wordt gepresenteerd met behulp van een flowschema voor diagnostisch onderzoek na een vruchtdood gebaseerd op vastgesteld waardevol onderzoek voor het aanwijzen van de doodsoorzaak door multidisciplinaire evaluatie van diagnostische procedures in het complete ZOBAS cohort van 1025 intra-uteriene antepartum vruchtdoden. Bij iedere vruchtdood werd een uitgebreid niet-selectief diagnostisch protocol uitgevoerd. De hoofdgroepen van doodsoorzaak waren placentaire pathologie (65.2%), aangeboren afwijkingen (4.8%), infectie (1.8%), anders (5.0%), terwijl in 23.2% de doodsoorzaak onbekend bleef. De meest waardevolle onderzoeken voor het vaststellen van de doodsoorzaak waren placentaonderzoek in 98%, obductie in 73% en cytogenetisch onderzoek in 29%. Foeto-maternale transfusie werd door een positieve Kleihauer-Betke test vastgesteld in 12% van de vrouwen. IgM antilichamen tegen virussen en Toxoplasmosis waren positief in 18% maar in slechts 1.8% ondersteunde placentaonderzoek of obductie een intra-uteriene infectie als doodsoorzaak. Diagnostisch onderzoek naar maternale ziekten werd alleen waardevol geacht als er een verdenking bestond op basis van de klinische voorgeschiedenis of de huidige graviditeit. Obductie, placentaonderzoek en cytogenetisch onderzoek zijn de basale diagnostische onderzoeken in een richtlijn voor alle vruchtdoden. Op basis van de resultaten of specifieke klinische karakteristieken wordt verder geïndividualiseerd sequentieel testen geadviseerd om zo onnodige onderzoeken en positieve onderzoeksresultaten te vermijden die de doodsoorzaak niet vaststellen.

List of publications

- Holm JP, Korteweg FJ. Alles over stuitligging. Boerhaave Commisie: Verloskunde voor de huisarts. nov. 1999:67-83.

- Korteweg FJ, Holm JP. Retrospective evaluation of the mode of delivery in term breech presentation. Ned. Tijdschrift voor Perinatale Geneeskunde. 2001;1:4-9.

- Erwich JJHM, Timmer A, Korteweg FJ. Classificatie van perinatale sterfte. Negende landelijke cursus kinderpathologie: Plotse dood. 2002:3-15.

- Korteweg FJ, Holm JP, Erwich JJHM. Zinnig onderzoek bij antepartum sterfte. Ned. Tijdschrift voor verloskundigen. 2003;5:226-231.

- Korteweg FJ, Holm JP, Erwich JJHM. Zinnig onderzoek bij antepartum sterfte. Beroepsvereniging voor Obstetrische en gynaecologische verpleegkundigen. 2004;2:6-8

- Korteweg FJ, Gordijn SJ, Timmer A, Erwich JJHM, Bergman KA, Bouman K, Ravisé JM, Heringa MP, Holm JP. The Tulip classification of perinatal mortality: introduction and multidisciplinary inter-rater agreement. BJOG. 2006;113:393-401

- Folkeringa N, Brouwer JLP, Korteweg FJ, Veeger NJGM, Erwich JJHM, Holm JP, van der Meer J. Reduction of high fetal loss rate by anticoagulant treatment during pregnancy in antithrombin, protein C or protein S deficient women. Br J Haematol. 2007;136:656-61

- Folkeringa N, Brouwer JLP, Korteweg FJ, Veeger NJGM, Erwich JJHM, van der Meer J. High risk of pregnancy-related venous thromboembolism in women with multiple thrombophilic defects. Br J Haematol. 2007;138:110-116

- Korteweg FJ, Erwich JJHM, Timmer A, van der Meer J, Ravisé JM, Holm JP. Meer kans op intra-uteriene vruchtdood bij extreem overgewicht. NTOG 2007;21:33-37.

- Korteweg FJ, Gordijn SJ, Timmer A, Holm JP, Ravisé JM, Erwich JJHM. A placental cause of intrauterine fetal death depends on the perinatal mortality classification system used. Placenta. 2008;29:71-80

- Korteweg FJ, Bouman K, Erwich JJHM, Timmer A, Veeger NJGM, Ravisé JM, Nijman TH, Holm JP. Cytogenetic analysis after evaluation of 750 fetal deaths: proposal for diagnostic workup. Obstet Gynecol. 2008;111:865-74.

- Gordijn SJ, Korteweg FJ, Erwich JJHM, Holm JP, van Diem M, Bergman K, Timmer A. A multilayered approach for the analysis of perinatal mortality using

different classification systems. Eur J Obstet Gynecol Reprod Biol. 2009;144:99-104

- Folkeringa N, Korteweg FJ, Veeger NJGM, Middeldorp S, Hamulyak K, Prins MH, Erwich JJ, Büller HR, van der Meer J. Thrombin activatable fibrinolysis inhibitor (TAFI) is not associated with fetal loss, a retrospective study. Thromb Res. 2009;123:511-514.

- Korteweg FJ, Erwich JJHM, Holm JP, Ravisé JM, van der Meer J, Veeger NJGM, Timmer A. Diverse placental pathologies as the main causes of fetal death. Obstet Gynecol. 2009;114:809-817.

- Flenady V, Silver RM, Incerpi M, Fretts RC, Pattinson R, Erwich JJ, Korteweg FJ, Frøen JF, Khong TY. Essential diagnostic work-up of stillbirths. In: Facchinetti F, Dekker GA, Saade G. Stillbirth: understanding and management. London: Informa Healthcare UK Ltd; 2009:1-28.

- Korteweg FJ, Folkeringa N, Brouwer JLP, Veeger NJGM, Erwich JJHM, Holm JP, Meer van der J. Fetal loss in women with hereditary deficiencies of antithrombin, protein C or protein S, and the contribution of cosegregation of other thrombophilic defects. Submitted

- Nijkamp JW, Korteweg FJ, Holm JP, Timmer A, Erwich JJHM, van Pampus MG. Subsequent pregnancy outcome after previous fetal death. Submitted

- Gordijn SJ, Korteweg FJ, Erwich JJHM, Holm JP, Ravisé JM, Nikkels PGJ, Veeger NJGM, Khong YT, Timmer A. Placental villus immaturity as an important cause of term fetal death. Submitted.

- Korteweg FJ, Erwich JJHM, Folkeringa N, Timmer A, Veeger NJGM, Ravisé JM, Holm JP, van der Meer J. New insight into thrombophilic defects in 750 couples with fetal death. Submitted

- Korteweg FJ, Erwich JJHM, Timmer A, van der Meer J, Ravisé JM, Veeger NJGM, Holm JP. Evaluation of 1025 fetal deaths; proposal for diagnostic work-up. Submitted

Dankwoord

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Mijn copromotor **dr. J.J.H.M. Erwich**. Beste Jan Jaap. Ik ben je dankbaar dat jij mij na mijn sollicitatiegesprek voor de ZOBAS studie geselecteerd hebt om dit uitdagende onderzoek op te zetten en uit te werken. Jij gaf mij het vertrouwen dat nodig was. Dit bleek ook uit de activiteiten, waar je mij als onderzoeker van de ZOBAS studie mee naar toenam of naar toe stuurde. Jij hebt mij veel geleerd op het gebied van de perinatale sterfte. Variërend van wetenschappelijk brainstormen,

kritisch maar ook praktisch inhoudelijk becommentariëren tot het onderhandelen over onderzoeksgelden of vriezers voor het opslaan van bloed. Als ik dreigde ten onder te gaan in logistieke problemen wist jij dat gelukkig altijd te relativeren. De congressen in het buitenland waren hoogtepunten in het promotietraject. Onze passie voor wedstrijdzeilen gaf interessante discussies over boten en snelheid. Misschien moeten we de theorie maar eens toetsen in de praktijk.

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De secretaresses die de administratieve zaken stroomlijnden **Anita Olinga, Simone Snijders** en in het bijzonder **Joke Breed** dank ik voor al hun inspanningen. Lieve Joke, met jou samenwerken was altijd erg prettig door je positieve en open houding. Geweldig al die ontbrekende gegevens die je hebt weten te achterhalen. Daarnaast ben ik **Simone Binnema** ook veel dank verschuldigd voor haar hulp in het begin van het ZOBAS traject toen er nog helemaal geen secretariële ondersteuning was. Lieve Simone, je was altijd bereid om mij naast je gewone werk te helpen, zelfs als dat betekende dat er 's avonds stickers moesten worden uitgedraaid. Daarnaast kon ik altijd mijn hart even luchten over onderzoeksperikelen.

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Lieve **Emmily en Juliëtte**, mijn kleine prinsesjes, jullie lach en armpjes om mij heen is puur levensgeluk.

Curriculum Vitae

Fleurisca Joyce Korteweg werd op 29 oktober 1975 in Delft geboren, als oudste uit een gezin van drie kinderen. Zij volgde het lagere schoolonderwijs in Rijswijk en Woking in Engeland. Het middelbare schoolonderwijs volgde zij op diverse plaatsen, te weten het Rijnlands Lyceum Cobham in Engeland, het Maerlant Lyceum in Den Haag en het Maartenscollege in Haren alwaar zij in 1994 haar VWO-examen behaalde.

De studie geneeskunde werd aan de Rijksuniversiteit te Groningen gevolgd. Gedurende haar studie werkte zij aan verschillende onderzoeksprojecten bij de Vakgroep Kindergeneeskunde van het Universitair Medisch Centrum Groningen o.l.v. dr. W.H. van Luijk, de Vakgroep Kindergeneeskunde van het Kalafong Ziekenhuis, Pretoria in Zuid Afrika o.l.v. dr. C.M.A. Bijleveld en dr. A. Grobler en de Vakgroep Obstetrie en Gynaecologie van het Universitair Medisch Centrum Groningen o.l.v. Prof. dr. J.P. Holm. Haar co-schappen in het noorden sloot zij af met een keuze co-schap Obstetrie en Gynaecologie in het Onze Lieve Vrouwe Gasthuis in Amsterdam.

Na het behalen van het artsexamen in 2001 was zij kort werkzaam als arts-assistent Obstetrie en Gynaecologie in het Kennemer Gasthuis in Haarlem (opleider dr. J.P. Lips). In 2002 begon zij met het opzetten van de ZOBAS studie (Zinnig Onderzoek Bij Antepartum Sterfte), haar promotieonderzoek o.l.v. Prof. dr. J.P. Holm en dr. J.J.H.M. Erwich bij de Vakgroep Obstetrie en Gynaecologie in het Universitair Medisch Centrum Groningen. Tevens was zij werkzaam als arts-assistent Obstetrie en Gynaecologie in dit ziekenhuis.

Op 1 oktober 2005 werd in het Universitair Medisch Centrum Groningen gestart met de opleiding Obstetrie en Gynaecologie in het opleidingscluster Groningen (opleiders: Prof dr. M.J. Heineman en Prof dr. M.J.E. Mourits). Van 1 oktober 2006 tot 1 oktober 2007 werd de opleiding onderbroken om een jaar full time aan haar wetenschappelijk onderzoek te werken, waarna de opleiding weer werd hervat. Vanaf juli 2010 zal zij haar opleiding tot gynaecoloog afronden in het Martini Ziekenhuis te Groningen (opleider: dr. A.J. van Loon). In 2005 is zij getrouwd met Rein Nieuwenhuis en samen hebben zij twee dochters Emmily (2006) en Juliëtte (2008).

Appendix 1

The following 50 Dutch hospitals participated in our national ZOBAS study.

Albert Schweitzer Hospital, Dordrecht; Alysis Zorggroep, Zevenaar; Amphia Hospital, Breda; Antonius Hospital, Sneek; Atrium Medical Centre, Heerlen; Bethesda Hospital, Hoogeveen; Bronovo Hospital, the Hague; Deventer Hospitals; Diakonessenhuis, Utrecht; Erasmus Medical Centre Rotterdam; Flevoziekenhuis, Almere; Gelre Hospitals, Apeldoorn; Gelre Hospitals, Zutphen; Groene Hart Hopsital, Gouda; Haga Hospital, the Hague; Hospital Amstelland, Amstelveen; Isala Klinieken, Zwolle; Kennemer Gasthuis, Haarlem; Lange Land Hospital, Zoetermeer; Leids University Medical Centre, Leiden; Maasstad Hopsital, Rotterdam; Martini Hospital, Groningen; Meander Medical Centre, Amersfoort; Medical Centre Alkmaar; Medical Centre Haaglanden Westeinde, the Hague; Medical Centre Leeuwarden; Medical Spectrum Twente, Enschede; Nij Smellinghe, Drachten; Orbis Medisch en Zorgconcern, Sittard; Rijnland Hospital, Leiderdorp; Rivierenland Hospital, Tiel; Rode Kruis Hospital, Beverwijk; Ruwaard van Putten Hospital, Spijkenisse; Scheperhospital, Emmen; Sint Elisabeth Hospital, Tilburg; Sint Franciscus Gasthuis, Rotterdam; Sint Lucas Andreas Hospital, Amsterdam; Slingeland Hospital, Doetinchem; Twee Steden hospital, Tilburg; University Medical Centre Groningen; Univeristy Medical Centre St. Radboud, Nijmegen; University Medical Centre Utrecht; VieCuri Medical Centre Noord Limburg, Venlo; Vlietland Hospital, Vlaardingen; Vrije University Medical Centre, Amsterdam; Walcheren Hospital, Vlissingen; Westfries Gasthuis, Hoorn; Wilhelmina Hospital, Assen; Zaans Medical Centre, Zaandam; ZGT, Hengelo;

Appendix 2

Onderzoeksnummer:		**ZOBAS**	

ZINNIG ONDERZOEK BIJ ANTEPARTUM STERFTE

1.1 Ziekenhuis _____

Kruis slechts één antwoord aan !

2 INCLUSIECRITERIA

2.1	Betreft het een éénlingzwangerschap	Nee ○ Ja ○
2.2	Zwangerschapsduur > 20 weken	Nee ○ Ja ○
2.3	IUVD vóór de partus vastgesteld	Nee ○ Ja ○

Alleen indien alle bovenstaande vragen met Ja zijn beantwoord, onderzoek voortzetten.

3 OVERZICHT UITSLAGEN DIAGNOSTIEK

Alle uitslagen van diagnostiek invullen!

Deel I ingevuld	Nee ○ Ja ○	Kopie obductie verslag	Nee ○ Ja ○
Deel II ingevuld	Nee ○ Ja ○	Kopie placenta verslag	Nee ○ Ja ○
Deel III ingevuld	Nee ○ Ja ○	Kopie MRI verslag	Nee ○ Ja ○
Deel IV ingevuld	Nee ○ Ja ○	Kopie Babygram verslag	Nee ○ Ja ○
Deel V ingevuld	Nee ○ Ja ○		

4 DOODSOORZAAK IUVD (VOLGENS U)

4.1 Werkdiagnose _____

_____

_____

4.2 Verdere diagnostiek nagelaten omdat: _____

_____

5 INSTRUCTIES

- ***Deel patiënteninformatie uit: noteer toestemming in status***
- ***Nieuwe IUVD aanmelden via e-mail: f.j.korteweg@og.azg.nl***
- ***Studieprotocol volledig invullen***
- ***Inzetten diagnostiek***
- ***Indien uitslagen bekend gaarne invullen.***

Bewaar het protocol in de status zodat bij de nacontrole alle gegevens kunnen worden ingevuld. Indien alle onderdelen zo volledig mogelijk zijn ingevuld s.v.p. het protocol + kopie van obductie en placentaverslag + eventueel kopieën van MRI of babygram verslag in de antwoordenvelop retour AZG.

BIJ VOORBAAT DANK VOOR UW TIJD EN MEDEWERKING

Onderzoeksnummer:		ZOBAS	

6 IDENTIFICATIE

6.1 Etiket ponsplaatje patiënte: vrouw

6.2 Etiket ponsplaatje partner patiënte: man

Onderzoeksnummer:		**ZOBAS**	
Deel I: Voorgeschiedenis en familieanamnese			

7 GEGEVENS PATIËNTE: VROUW - PERSOONLIJKE GEGEVENS

7.1 Etnische origine ○ Kaukasisch ○ Hindoestaans ○ Negroïde ○ Mediterraan ○ Aziatisch ○ Overig

Indien Overig, specificeer: _____

7.2 Burgerlijke staat ○ Alleenstaand ○ Samenwonend ○ Gehuwd

7.3 Beroep _____

7.4 Hoogst genoten opleiding (voor specificatie zie bijlage 1: tabel 1)

○ Lagere school
○ Lager beroepsonderwijs
○ Algemeen middelbaar onderwijs
○ Middelbaar beroepsonderwijs
○ Algemeen voortgezet onderwijs
○ Hoger beroepsonderwijs
○ Wetenschappelijk onderwijs

8 GEGEVENS PATIËNTE: VROUW - ALGEMENE VOORGESCHIEDENIS

8.1 Hypertensie ○ Ja ○ Nee ○ Onbekend

8.2 Systeemziekten ○ Ja ○ Nee ○ Onbekend

indien Ja, specificeer:

8.2.1 Reuma ○ Ja ○ Nee ○ Onbekend

8.2.2 SLE ○ Ja ○ Nee ○ Onbekend

8.2.3 Sclerodermie ○ Ja ○ Nee ○ Onbekend

8.2.4 Overige ○ Ja ○ Nee

Indien Ja, specificeer: _____

8.3 Diabetes mellitus ○ Ja ○ Nee ○ Onbekend

8.4 Schildklierpathologie ○ Ja ○ Nee ○ Onbekend

8.5 Bloedtransfusie ○ Ja ○ Nee ○ Onbekend

8.6 Erfelijke trombofilie ○ Ja ○ Nee ○ Onbekend

Indien Ja, specificeer: _____

8.7 Veneuze trombose/ longembolie ○ Ja ○ Nee ○ Onbekend

Indien Ja, jaartal(len): _____

8.8 Chronische nierziekten ○ Ja ○ Nee ○ Onbekend

8.9 Cholestase ○ Ja ○ Nee ○ Onbekend

8.10 Overige ○ Ja ○ Nee

Indien Ja, specificeer: _____

9 GEGEVENS PATIËNTE: VROUW - FAMILIE ANAMNESE

9.1 Consanguiniteit ○ Ja ○ Nee ○ Onbekend

9.2 1e graads (ouders, zussen, broers) veneuze trombose/ longembolie ○ Ja ○ Nee ○ Onbekend

9.3 Erfelijke trombofilie ○ Ja ○ Nee ○ Onbekend

Indien Ja, specificeer: _____

9.4 1e graads (ouders, zussen, broers) IUVD ○ Ja ○ Nee ○ Onbekend

Onderzoeksnummer:		**ZOBAS**	

Deel I: Voorgeschiedenis en familieanamnese

10 GEGEVENS PATIËNTE: VROUW - OBSTETRISCHE VOORGESCHIEDENIS

10.1	Pariteit	○ Nulli ○ Primi ○ Multi ○ Grande multi (>4)
10.2	Recidiverende abortus (2 of meer)	○ Ja ○ Nee ○ Onbekend
10.3	IUVD	○ Ja ○ Nee ○ Onbekend
10.4	Pre-eclampsie	○ Ja ○ Nee ○ Onbekend
10.5	Macrosomie	○ Ja ○ Nee ○ Onbekend
10.6	Foetale groeivertraging	○ Ja ○ Nee ○ Onbekend
10.7	Diabetes	○ Ja ○ Nee ○ Onbekend
10.8	Diabetus gravidarum	○ Ja ○ Nee ○ Onbekend
10.9	Bloedgroepantagonisme	○ Ja ○ Nee ○ Onbekend
10.10	Overig	○ Ja ○ Nee
	Indien Ja, specificeer:	_____

11 GEGEVENS PARTNER PATIËNTE: MAN - PERSOONLIJKE GEGEVENS

11.1	Beroep	_____

12 GEGEVENS PARTNER PATIËNTE: MAN - ALGEMENE VOORGESCHIEDENIS

12.1	Erfelijke trombofilie	○ Ja ○ Nee ○ Onbekend
	Indien Ja, specificeer:	_____
12.2	Veneuze trombose/ longembolie	○ Ja ○ Nee ○ Onbekend
	Indien Ja, jaartal(len):	_____

13 GEGEVENS PARTNER PATIËNTE: MAN - FAMILIE ANAMNESE

13.1	1e graads (ouders, zussen, broers) veneuze trombose/ longembolie	○ Ja ○ Nee ○ Onbekend
13.2	Erfelijke trombofilie	○ Ja ○ Nee ○ Onbekend
	Indien Ja, specificeer:	_____

Onderzoeksnummer:		**ZOBAS**	

Deel II: Huidige Graviditeit

14 ALGEMEEN

14.1 Ontstaan zwangerschap ○ Spontaan ○ Ovulatie inductie ○ IVF/ ICSI

14.2 Antenatale diagnostiek ○ Ja ○ Nee

Indien Ja:

14.2.1 Vlokkentest ○ Ja ○ Nee

14.2.2 Vruchtwaterpunctie ○ Ja ○ Nee

Indien Ja voor één van de testen, uitslag: _____

14.3 Echo

14.3.1 Polyhydramnion ○ Ja ○ Nee

14.3.2 Oligohydramnion ○ Ja ○ Nee

14.3.3 Macrosomie ○ Ja ○ Nee

14.3.4 IUGR ○ Ja ○ Nee

14.3.5 Overig ○ Ja ○ Nee

Indien Ja, specificeer: _____

14.4 Maternaal gewicht en lengte

14.4.1 Eerste bekende gewicht kg |__|__|__|,|__| bij AD weken+dagen |__|__| + |__|

14.4.2 Laatste bekende gewicht kg |__|__|__|,|__| bij AD weken+dagen |__|__| + |__|

14.4.3 Lengte cm |__|__|__|,|__|

14.5 Zwangerschapscomplicaties

14.5.1 Diabetes ○ Ja ○ Nee

14.5.2 Hypertensie ○ Ja ○ Nee

14.5.3 Pre(eclampsie) ○ Ja ○ Nee

14.5.4 HELLP ○ Ja ○ Nee

14.5.5 Positieve dyscongruentie ○ Ja ○ Nee

14.5.6 Negatieve dyscongruentie ○ Ja ○ Nee

14.5.7 Bloedverlies in zwangerschap ○ Ja ○ Nee

14.5.8 Bloedgroepantagonisme ○ Ja ○ Nee

14.5.9 Trauma ○ Ja ○ Nee

14.5.10 Overig ○ Ja ○ Nee

Indien Ja, specificeer: _____

15 ZWANGERSCHAPSDUUR

15.1 Zekere termijn ○ Ja ○ Nee

15.2 AD bij vaststellen IUVD weken+dagen |__|__| + |__|

15.3 AD bij partus weken+dagen |__|__| + |__|

15.4 Datum partus dd mm jjjj |__|__|__|__|2|0|0|__|

Onderzoeksnummer:		**ZOBAS**	

Deel II: Huidige Graviditeit

16 MEDICATIE

16.1 Medicatie gebruik ○ Ja ○ Nee

Indien Ja, specificeer:

16.1.1 Immunosuppresiva ○ Ja ○ Nee

16.1.2 Thyreostatica ○ Ja ○ Nee

16.1.3 Insuline ○ Ja ○ Nee

16.1.4 Anti-epileptica ○ Ja ○ Nee

16.1.5 Anti-hypertensiva ○ Ja ○ Nee

16.1.6 Corticosteroïden ○ Ja ○ Nee

16.1.7 Psychofarmaca ○ Ja ○ Nee

16.1.8 Overig ○ Ja ○ Nee

Indien Ja, specificeer: _____

16.2 Foliumzuur gebruikt ○ Ja ○ Nee

17 DRUGSGEBRUIK

17.1 Drugsgebruik ○ Ja ○ Nee ○ Onbekend

Indien Ja, specificeer:

17.1.1 Heroïne ○ Ja ○ Nee

17.1.2 Cocaïne ○ Ja ○ Nee

17.1.3 Methadon ○ Ja ○ Nee

17.1.4 Hasj ○ Ja ○ Nee

18 BLOOTSTELLING AAN ROOK

Rookgedrag wordt uitgedrukt in aantal sigaretten per dag.

18.1 Actief ○ Nee ○ <5 ○ 5-10 ○ 11-20 ○ >20 ○ Onbekend

18.2 Passief ○ Nee ○ <5 ○ 5-10 ○ 11-20 ○ >20 ○ Onbekend

Indien *niet Nee*, specificeer bron ○ Partner ○ Anders

19 ALCOHOL GEBRUIK

19.1 Alcohol gebruik ○ Ja ○ Nee ○ Onbekend

Indien Ja, eenheden per dag |__|__|

Onderzoeksnummer:		**ZOBAS**	
		Deel III: Maternaal Onderzoek	

20 DEEL III: MATERNAAL BLOEDONDERZOEK

Antepartum of ten tijde van inleiding inzetten

20.1	Bloedgroup	○ A ○ B ○ AB ○ O
20.2	Rhesus faktor	○ Positief ○ Negatief
20.3	Hemoglobine	mmol/L __,_
20.4	Hematokriet	L/L _,__
20.5	Thrombocyten	10^9/L ___
20.6	C-reactive protein	mg/L __,_
20.7	Bilirubine totaal	µmol/L ___,_
20.8	Urinezuur	mmol/L _,__
20.9	Ureum	mmol/L __,_
20.10	Kreatinine	µmol/L ___
20.11	ASAT	U/L ___
20.12	ALAT	U/L ___
20.13	LDH	U/L ___
20.14	GGT	U/L ___
20.15	Kleihauer-Betke	○ Positief ○ Negatief
	Indien Positief:	ml ___
20.16	TSH	mU/L __,__
20.17	Vrij T4	pmol/L ___,_
20.18	Glucose	mmol/L __,_
20.19	HbA1c	% __,_
20.20	Hb electroforese Hb-pathie: (indien verdenking):	○ Ja ○ Nee
	Indien Ja, welke:	_____
20.21	ANF	○ Positief ○ Negatief
20.22	Homocysteïne	µmol/L ___,_
20.23	Plasma anticardiolipines IgG en IgM antilichamen	○ Positief ○ Negatief
20.24	Irregulaire antilichamen	○ Positief ○ Negatief
	Indien Positief, welke:	_____
20.25	HIV bekend?	○ Ja ○ Nee
	Indien Ja:	○ Positief ○ Negatief

Onderzoeksnummer:		**ZOBAS**	
		Deel III: Maternaal Onderzoek	

21 VIRAAL EN BACTERIEEL BLOEDONDERZOEK MOEDER

Antepartum of ten tijde van inleiding inzetten

21.1	Toxoplasmose	IgG	○ Positief	○ Negatief	IgM	○ Positief	○ Negatief
21.2	Rubella	IgG	○ Positief	○ Negatief	IgM	○ Positief	○ Negatief
21.3	Cytomegalie	IgG	○ Positief	○ Negatief	IgM	○ Positief	○ Negatief
21.4	Herpes	IgG	○ Positief	○ Negatief	IgM	○ Positief	○ Negatief
21.5	Parvo B19	IgG	○ Positief	○ Negatief	IgM	○ Positief	○ Negatief
21.6	Lues					○ Positief	○ Negatief
21.7	HbsAg					○ Positief	○ Negatief
21.8	Varicella zoster					○ Positief	○ Negatief

22 MATERNAAL EN PATERNAAL STOLLINGSONDERZOEK

Antepartum of ten tijde van inleiding inzetten

Bloed voor maternaal stollingsonderzoek:

- ***oranje ZOBAS stickers: noteer gegevens, sticker 4 citraatbuizen (5ml) met oranje ZOBAS stickers ® bloed afnemen: buizen goed vol laten lopen***

Bloed voor paternaal stollingsonderzoek:

- ***groene ZOBAS stickers: noteer gegevens, sticker 4 citraatbuizen (5ml) met groene ZOBAS stickers ® bloed afnemen: buizen goed vol laten lopen***

Plak op blauwe labinstructievel: etiket ponsplaatje moeder en vader + oranje ingevulde labsticker moeder + groene ingevulde labsticker vader

Stuur 8 citraatbuizen naar locaal lab + blauwe ZOBAS labinstructies + overige stickers

23 OVERIG ONDERZOEK

23.1	Urinesediment (stick): nitriet	○ Positief	○ Negatief
23.2	Urine: Chlamydia PCR	○ Positief	○ Negatief
23.3	Introïtus anuskweek: β hemolytische streptokok	○ Positief	○ Negatief

Onderzoeksnummer:		**ZOBAS**	

Deel IV: Onderzoek Kind

24 UITWENDIG ONDERZOEK KIND

24.1	Geslacht	○ Man ○ Vrouw ○ Onduidelijk
24.2	Maceratie aanwezig?	○ Ja ○ Nee
	Indien Ja, maceratiegraad (voor specificatie zie bijlage 1: tabel 2)	○ 1 ○ 2 ○ 3
24.3	Gewicht	g ____
24.4	Maten (alleen indien geen toestemming voor obductie)	
24.4.1	Schedelomtrek	cm ____
24.4.2	Kruin-stuitlengte	cm ____
24.4.3	Kruin-hiellengte	cm ____
24.4.4	Borstomtrek t.h.v tepels	cm ____
24.4.5	Omtrek buik t.h.v. navel	cm ____
24.4.6	Voetlengte	cm ____
24.5	Meconium	○ Ja ○ Nee
24.6	Navelstrengomstrengeling	○ Ja ○ Nee
	Indien Ja, aantal keer:	__

25 DYSMORFE KENMERKEN

25.1	Dysmorfe kenmerken aanwezig?	○ Ja ○ Nee

Indien Ja, documenteren (eventueel met foto's)

25.2	Hoofd / hals	
25.3	Thorax	
25.4	Abdomen	
25.5	Uitwendig geslacht	
25.6	Perineum	
25.7	Rugzijde	
25.8	Extremiteiten	

26 AANVULLEND ONDERZOEK KIND

Viraal en bacterieel onderzoek kind direct post partum inzetten.

26.1	Orofarynxkweek	○ Geen groei ○ Groei
	Indien Groei, specificeer:	

Onderzoeksnummer:		**ZOBAS**	

Deel IV: Onderzoek Kind

27 NAVELSTRENGBLOED

27.1	Navelstrengbloed verkregen?					○ Ja ○ Nee
27.2	Hemoglobine					mmol/L [,] , [ ]
27.3	Reticulocyten					$^{0}/_{00}$ [,]
27.4	Bloedgroup					○ A ○ B ○ AB ○ O
27.5	Rhesus faktor					○ Positief ○ Negatief
27.6	Coombs					○ Positief ○ Negatief
27.7	Bilirubine					µmol/L [,] , [ ]
27.8	LDH					U/L [, ,]
27.9	CPK (creatininephosphokinase)					U/L [, ,]
27.10	Toxoplasmose	IgG	○ Positief	○ Negatief	IgM	○ Positief ○ Negatief
27.11	Rubella	IgG	○ Positief	○ Negatief	IgM	○ Positief ○ Negatief
27.12	Cytomegalie	IgG	○ Positief	○ Negatief	IgM	○ Positief ○ Negatief
27.13	Herpes	IgG	○ Positief	○ Negatief	IgM	○ Positief ○ Negatief
27.14	Parvo B19	IgG	○ Positief	○ Negatief	IgM	○ Positief ○ Negatief
27.15	Lues					○ Positief ○ Negatief

28 KARYOTYPERING

28.1	Karyotypering verricht?					○ Ja ○ Nee
28.2	Aard materiaal	○ Chorion villus biopsie	○ Vruchtwater	○ Navelstreng	○ Foetaal bloed	
		○ Fascia lata	○ Pericard	○ Kraakbeen	○ Anders	
	Indien Anders, specificeer:					
28.3	Uitslag					○ Mislukt ○ Normaal ○ Afwijkend
	Indien Normaal of Afwijkend, specificeer:					

29 OBDUCTIE

29.1 Ouders geven toestemming voor obductie? ○ Ja ○ Nee

Indien Ja, Zobas pathologierichtlijn meesturen met obductieaanvraag.

Indien Nee, MRI of babygram aanvragen.

Onderzoeksnummer:		**ZOBAS**	
Deel V: Onderzoek Placenta			

30 MACROSCOPIE PLACENTA

30.1 Placenta gewicht (inclusief vliezen en navelstreng) g |__|__|__|__|

30.2 Vliezen _____

_____

_____

30.3 Navelstreng

30.3.1 Lengte cm |__|__|.|__|

30.3.2 Insertie ○ Centraal ○ Paracentraal ○ Velamenteus

30.3.3 Aantal vaten ○ 2 ○ 3

30.3.4 Echte knoop ○ Ja ○ Nee

30.3.5 Trombus ○ Ja ○ Nee

30.3.6 Uitgezakt ○ Ja ○ Nee

30.3.7 Overig _____

_____

_____

30.4 Foetale zijde placenta ○ Normaal ○ Afwijkend

Indien Afwijkend, specificeer: _____

_____

30.5 Maternale zijde placenta ○ Normaal ○ Afwijkend

Indien Afwijkend, specificeer: _____

_____

31 MICROBIOLOGISCH ONDERZOEK PLACENTA

31.1 Placentakweek ○ Geen groei ○ Groei

Indien Groei, specificeer: _____

31.2 Listeria ○ Positief ○ Negatief

32 INSTRUCTIES PLACENTA

Placenta naar pathologie: indien geen obductie toestemming stuur dan nu Zobas pathologierichtlijn mee.

Onderzoeksnummer:		ZOBAS	
Deel V: Onderzoek Placenta			

33 AFRONDING ONDERZOEK

Post partum contrôle: alle uitslagen diagnostiek invullen!

Indien uitslagen kompleet: Stuur in antwoordenvelop retour AZG:

- *Ingevulde Zobas protocol*
- *Kopie obuctie en placentaverslag*
- *Eventueel kopie MRI of babygramverslag*

GA TERUG NAAR VOORBLAD STUDIEPROTOCOL

Hartelijk dank voor uw tijd en medewerking!

		ZOBAS	
Bijlage I			

TABEL 1: HOOGST GEVOLGDE OPLEIDING

Lagere School	Lager algemeen onderwijs/ basisonderwijs of een gedeelte hiervan
Lager Beroepsonderwijs	b.v. LTS, LHNO, LEAO, detailhandelschool, lager land- en tuinbouwonderwijs
Algemeen Middelbaar Onderwijs	bv: LAVO, VGLO, ULO, MULO, MAVO-3, MAVO-4, 3-jarig HBS, middenschool
Middelbaar Beroepsonderwijs	bv: MEAO, MTS, UTS, MBA, NIMA-A, horecaschool
Algemeen Voortgezet Onderwijs	HBS, MMS, HAVO, VWO
Hoger Beroepsonderwijs	bv: HTS, HEAO, Sociale academie, HBO-V, Kweekschool, NIMA B/C

TABEL 2: MACERATIEGRADEN

Maceratie: veranderingen die ontstaan in foetaal weefsel na IUVD

Classificatie	Tijd na dood	Veranderingen
Geen maceratie	< 6 - 8 uur	geen veranderingen
GRAAD 1	8 - 24 uur	loslatende huid
	24 - 28 uur	bullae, vocht verliezende gebieden
GRAAD 2	48 uur	diffuse roodheid
	4 - 5 dagen	moulage van schedelbeenderen
GRAAD 3	6 - 7 dagen	omhoog komen van schedelbeenderen
	8 - 10 dagen	bruine huid
	3 weken	grijze gerimpelde huid

Appendix 3

Flowschema

Zinnig onderzoek bij antepartum sterfte

INCLUSIECRITERIA:

Alle eenling zwangerschappen van > 20 weken zwangerschapsduur waarbij een IUVD ante partum is vastgesteld

DEEL PATIËNTENINFORMATIE UIT: NOTEER TOESTEMMING IN STATUS
- nieuwe IUVD aanmelden via email (locale coördinator): f.j.korteweg@og.azg.nl

Invullen

- **Deel I: voorgeschiedenis en familieanamnese**
- **Deel II: huidige graviditeit**

Inzetten

- **Deel III: maternaal bloed (ten tijde van inleiding):** Hb, Ht, Trombocyten, CRP, Bili totaal, Urinezuur, Ureum, Kreatinine, ASAT, ALAT, LDH, GGT, Kleihauer-Betke, TSH, FT4, glucose, HbA1c, Hb electroforese Hb-pathie (indien verdenking), ANF, homocysteine (niet nuchter), plasma anticardiolipines IgG en IgM antilichamen, Toxoplasmose IgG/IgM, Rubella IgG/IgM, Cytomegalie IgG/IgM, Herpes IgG/IgM, Lues: TPHA, HbsAg, Varicella Zoster IgG/IgM, Parvo B19 IgG/IgM

- **Bloed voor maternaal stollingsonderzoek:** oranje ZOBAS stickers: noteer gegevens, sticker 4 citraatbuizen (5ml) met oranje ZOBAS stickers → bloed afnemen: buizen goed vol laten lopen
- **Bloed voor paternaal stollingsonderzoek:** vader poliklinisch inschrijven groene ZOBAS stickers: noteer gegevens, sticker 4 citraatbuizen (5ml) met groene ZOBAS stickers → bloed afnemen: buizen goed vol laten lopen

- Plak op blauwe ZOBAS-labinstructievel etiket ponsplaatje moeder en vader + oranje ingevulde labsticker moeder + groene ingevulde labsticker vader
- Stuur 8 citraatbuizen naar lab + blauwe ZOBAS-labinstructies + overige stickers

UMCG stollingsbepalingen: plasma spiegels Antithrombine, proteine C, totaal en vrij proteine S, von Willebrand factor, Factor V Leiden, Prothrombin G20210A mutatie, lupus anticoagulant

- Urinesediment (stick)
- Urine: Chlamydia PCR
- Introïtus/anuskweek: ß-hemolytische streptokok

Invullen

- **Deel IV: onderzoek kind**

↓

Inzetten

- Orofarynxkweek kind
- **Navelstrengbloed kind:** Hb, Reticulocyten, Bloedgroep rhesus, Coombs, Bilirubine, LDH, CPK, Toxoplasmose IgG/IgM, Rubella IgG/IgM, Cytomegalie IgG/IgM, Herpes IgG/IgM, Parvo B19 IgG/IgM, Lues IgG/IgM

↓

- **Karyotypering kind:** -chorion villus biopsie of vruchtwater of
  -navelstreng of fascia lata of
  -oorkraakbeen of navelstrengbloed

↓

OBDUCTIE

Toestemming → ✎ Vul obductieaanvraag in + stuur ZOBAS pathologierichtlijn mee

Geen toestemming → ✎ **MRI of babygram** aanvragen (< 24 uur)

Invullen

- Deel V: macroscopisch onderzoek placenta

↓

Inzetten

- Placentakweek
- Placenta voor Pathologie
(indien geen obductie: stuur dan nu ZOBAS pathologierichtlijn mee)

TIJDENS PARTUS EN BIJ POST PARTUM CONTROLE:

Invullen op cheklist

-uitslagen: - maternaalbloed
- urine: sediment, chlamydia PCR, introïtus/anuskweek, orofarynxkweek
- navelstrengbloed, karyotypering, placentakweek

↓

Opsturen naar UMCG

- volledig ingevulde ZOBAS studieprotocol
- kopie obductie en placentaverslag

Appendix 4

ZOBAS PATHOLOGY PROTOCOL
ZINNIG ONDERZOEK BIJ ANTEPARTUM STERFTE
RICHTLIJN OBDUCTIE EN PLACENTA ONDERZOEK

Op basis van specifieke informatie en/ of "klinische blik" van de patholoog kan afhankelijk van de situatie aangepast, aanvullend onderzoek worden verricht. Voor achtergrond informatie raadpleeg de "Toelichting op de richtlijn obductie en placenta onderzoek".

Onderzoeksnummer ZOBAS

OBDUCTIE

Uitwendig onderzoek:

Metingen:
- -Lichaamsgewicht
- -Kruin-stuitlengte
- -Kruin-hiellengte
- -Schedelomtrek
- -Borstomvang
- -Buikomvang
- -Voetlengte
- -Femurlengte

Röntgen onderzoek (op indicatie, in geval van congenitale afwijkingen):
- -"Total body" opname, bijvoorkeur voorachterwaarts en lateraal

Dysmorfe kenmerken en / of andere afwijkingen:
- -Documenteren, bijvoorkeur met behulp van foto's
- -Aanvullende röntgen opnamen

Inwendig onderzoek:

Macroscopie:
- -Inspectie van borst-, buik-, en schedelholte
- -Wegen van organen (thymus, hart, longen, lever, nieren, bijnieren, milt, hersenen)
- -Systematische beschrijving van schedel, ribben en organen (thymus, hart, bovenste luchtwegen, longen, lever, milt, pancreas, darmen, bijnieren, nieren, ureteren, blaas, gonaden)

Microscopie:

-Van alle organen (inclusief navelstreng insertie) tenminste een sample in paraffine. Indien geen toestemming voor totale obductie, is het nemen van biopten (in overleg) te overwegen.

Placenta: Dient in alle gevallen deel uit te maken van het onderzoek

Aanvullend onderzoek:

-Veilig stellen materiaal of in geval van congenitale afwijkingen chromosomen onderzoek (pericard en kraakbeen)
-Op indicatie DNA flowcytometrisch onderzoek
-Op indicatie vries materiaal lever, milt, spier, ...
-Op indicatie microbiologisch onderzoek (longen, milt, ...)

Obductie verslag: Onderzoeksnummer ZOBAS

-Systematische beschrijving van uitwendig- en inwendig onderzoek, inclusief maten en gewichten
-Systematische beschrijving van röntgen onderzoek en overig aanvullend onderzoek
-Placenta onderzoek (minimaal gewicht en conclusie)
-Geslacht
-Groei en ontwikkeling
-Congenitale afwijkingen
-Andere afwijkingen
-Tijdstip intra-uteriene vruchtdood
-Acute asfyxie
-Chronische stress
-Doodsoorzaak
-Klinisch-pathologische concordantie
-Herhalingsrisico

PLACENTA ONDERZOEK

Macroscopie:

-Gewicht na afname van vliezen en navelstreng
-Afmetingen (maximale- en minimale diameter en dikte)
-Navelstreng: lengte, aantal windingen, coiling-index, insertie, aantal vaten, ...
-Vliezen: plaats vliesscheur, ...
-Choriaal plaat: .....
-Parenchym: .....
-Basale plaat: .....

Microscopie:

-Navelstreng foetale zijde en maternale zijde (niet < 5 cm insertie):
aantal vaten, …..

-Vliezenrol vanuit de vliesscheur naar placentarand: …..

-Tenminste 3 samples van macroscopisch niet afwijkend placenta parenchym (centrale deel cotyledon, inclusief choriaal plaat met amnion en basale plaat) + sample(s) macroscopisch afwijkend placenta parenchym: ontwikkeling en vascularisatie van de chorion villi , ….

Aanvullend onderzoek (op indicatie):

-DNA flowcytometrisch onderzoek
-Microbiologisch onderzoek

_____

_____

_____

Placenta verslag:

-Onderzoeksnummer ZOBAS
-Systematische beschrijving van macroscopie en microscopie
-Bespreking macroscopie en microscopie in relatie tot klinische gegevens
-Herhalingsrisico

TOELICHTING OP DE PATHOLOGIE RICHTLIJN ZINNIG ONDERZOEK BIJ ANTEPARTUM STERFTE **TOELICHTING** OP DE **RICHTLIJN** OBDUCTIE EN PLACENTA ONDERZOEK

OVERLIJDEN

Ziekenhuis: _____

Naam kind: _____

Geboortedatum: _ _- _ _- _ _ _ _

Tijdstip: _ _: _ _ uur

Datum postmortaal onderzoek: _ _- _ _- _ _

Onderzoeksnummer ZOBAS

OBDUCTIE: eigen referentie nummer: _____

Uitwendig onderzoek:

Regressieve veranderingen passend bij intra-uteriene vruchtdood:
zie bijlage I, tabel 1

Metingen: zie bijlage I, tabel 2

Dysmorfe kenmerken of andere afwijkingen: documenteren (foto's)

☐ nee ☐ jongen
☐ meisje
☐ ja, nl:

Hoofd/ hals: _____

_____

Thorax: _____

_____

Abdomen: _____

_____

Uitwendig geslacht: _____

_____

Perineum:

Rugzijde:

Extremiteiten:

Inwendig onderzoek:

Macroscopie:

-Inspectie van borst-, buik-, en schedelholte

-Wegen van organen

-Systematische beschrijving van schedel, ribben en organen

Wegen van organen: zie bijlage I, tabel 2

Ontwikkeling van de hersenen:

☐ normaal voor de amenorrhoeduur

☐ voorlopend op de amenorrhoeduur

☐ achterlopend op de amenorrhoeduur

Performance of the perinatal autopsy (figure 3.8) in Perinatal Pathology, second edition. Wigglesworth (ed). WB Saunders Company, 1996

Structurele- of andere afwijkingen:

☐ nee

☐ ja, nl:

Hals/ thorax:

Abdomen:

Hersenen:

Microscopie:
Van alle organen (inclusief navelstreng insertie) tenminste een sample in paraffine (indien geen toestemming voor totale obductie is het nemen van biopten in overleg te overwegen). **Regressieve veranderingen passend bij intra-uteriene vruchtdood**: zie bijlage I, tabel 1

Ontwikkeling van longen en nieren in relatie tot de amenorrhoeduur (AD) :

Longen : ☐ pseudoglandulair stadium (7-17 weken AD)
☐ canaliculair stadium (17-24 weken AD)
☐ sacculair stadium (24-32 weken AD)
☐ alveolair stadium (≥32 weken AD)

Nieren: ☐ nefrogene zone ☐ nee (≥ 36 weken AD)
☐ ja ☐ incompleet (34 AD)
☐ compleet
☐ .....lagen glomeruli in de cortex, passend bij .....weken AD

The respiratory system and the kidneys and urinary tract in Perinatal Pathology, second edition. Wigglesworth (ed). WB Saunders Company, 1996.

Structurele- of andere afwijkingen:
☐ nee
☐ ja, nl:

Hals/ thorax: _____

_____

Abdomen: _____

_____

Hersenen: _____

_____

Aanvullend onderzoek:

Röntgen onderzoek (total body, bijvoorkeur voorachterwaarts en lateraal):
☐ niet verricht
☐ verricht

Ossificatie:
☐ normaal voor de opgegeven amenorrhoeduur
☐ achterlopend op de opgegeven amenorrhoeduur
☐ voorlopend op de opgegeven amenorrhoeduur

Structurele afwijkingen:
☐ nee
☐ ja, nl:

Veilig stellen materiaal (pericard en kraakbeen):
☐ niet verricht
☐ verricht: ☐ mislukt;
☐ gelukt

In geval van congenitale afwijkingen chromosomen onderzoek (pericard en kraakbeen):
☐ niet verricht
☐ verricht, uitslag: ☐ mislukt; ☐ normaal mannelijk karyotype; ☐ normaal vrouwelijk karyotype; ☐ afwijkend karyotype, nl:

_____

_____

_____

Op indicatie DNA flowcytometrisch onderzoek:
☐ niet verricht
☐ verricht, uitslag: 0 diploid; 0 triploid; 0.....

Op indicatie microbiologisch onderzoek (longen, milt, ...):
☐ niet verricht
☐ verricht, uitslag:

_____

_____

_____

DEFINITIEVE OBDUCTIE BEVINDINGEN:

Tijdstip van intra-uterien overlijden
- ☐ < 6 uur voor de geboorte
- ☐ < 24 uur voor de geboorte
- ☐ < 2 dagen voor de geboorte
- ☐ 2-6 dagen voor de geboorte
- ☐ ≥ 7 dagen voor de geboorte
- ☐ ≥ 14 dagen voor de geboorte
- ☐ ≥ 4 weken voor de geboorte
- ☐ ≥ 8 weken voor de geboorte

Foetale groei
- ☐ small for gestational age
  - ☐ asymmetrisch
  - ☐ symmetrisch
- ☐ appropriate for gestational age
- ☐ large for gestational age

Acute asfyxie
- ☐ nee
- ☐ ja

Chronische stress
- ☐ nee
- ☐ ja

Congenitale afwijkingen:
- ☐ nee
- ☐ ja
  - ☐ chromosomale afwijking
  - ☐ syndromale afwijking
- ☐ anders, nl

_____

_____

_____

Afwijkingen passend bij infectie
- ☐ nee
- ☐ ja

Afwijkingen passend bij (ernstige) foetale anemie
- ☐ nee
- ☐ ja

Hydrops foetalis eci
- ☐ nee
- ☐ ja

Intra-foetale verbloeding
- ☐ nee
- ☐ ja

_____

_____

_____

Onderzoeksnummer ZOBAS

PLACENTA ONDERZOEK: eigen referentie nummer: .....

Macroscopie:

Gewicht:	zie bijlage I, tabel 2
Afmetingen:	zie bijlage I, tabel 2
Placenta circumvallata:	☐ nee
	☐ ja
Bijplacenta:	☐ nee
	☐ ja
Meconium:	☐ nee
	☐ ja

Navelstreng:

Insertie:	centraal / paracentraal / marginaal / velamenteus
Lengte:	zie bijlage I, tabel 2
Aantal windingen:	zie bijlage I, tabel 2
Aantal vaten:	☐ 2
	☐ 3
Echte knopen:	☐ nee
	☐ ja
Thrombose:	☐ nee
	☐ ja
Bloeding:	☐ nee
	☐ ja
Oedeem:	☐ nee
	☐ ja

_____

_____

_____

Vliezen:	☐ normaal
	☐ afwijkend, nl:
Vasa praevia	☐ nee
	☐ ja

_____

_____

_____

Foetale zijde: ☐ normaal
☐ afwijkend, nl:
Thrombose: ☐ nee
☐ ja
Bloeding: ☐ nee
☐ ja, diameter .....cm

Maternale zijde: ☐ normaal
☐ afwijkend, nl:
Bloeding zonder delle: ☐ nee
☐ ja, diameter .....cm, dikte ..... cm
Bloeding met delle: ☐ nee
☐ ja, diameter .....cm, dikte ..... cm
Randbloeding zonder delle ☐ nee
☐ ja, diameter .....cm, dikte ..... cm
Randbloeding met delle ☐ nee
☐ ja, diameter .....cm, dikte..... cm

Parenchym: ☐ normaal
☐ afwijkend, nl:
Infarcering ☐ nee
☐ ja ☐perifeer ☐centraal ☐oud
☐ recent ☐<10% ☐10-20% ☐20-50%
☐> 50%
Fibrine depositie ☐ nee
☐ ja ☐rand ☐subchoriaal ☐basaal
☐ diffuus ☐< 10% ☐10-20%
☐ 20-50% ☐> 50%
Intervilleuze thrombose ☐ <3cm ☐>3 cm ☐multiple
☐ subchoriaal ☐< 10% ☐10-20%
☐ 20-50% ☐> 50%e

Microscopie:

Navelstreng foetale zijde en maternale zijde (niet < 5 cm vanaf insertie):

☐ normaal
☐ afwijkend, nl:

Funiculitis:
☐ nee
☐ ja

_____

_____

_____

Vliezenrol (vanuit de vliesscheur naar de placentarand):

☐ normaal
☐ afwijkend, nl:

Acute chorioamnionitis
☐ nee
☐ ja

_____

_____

_____

Tenminste 3 samples van macroscopisch niet afwijkend placentaparenchym (centrale deel cotyledon, inclusief choriaal plaat met amnion en basale plaat) + sample(s) macroscopisch afwijkend placenta parenchym:

☐ normaal
☐ afwijkend, nl:

Regressieve afwijkingen passend bij intra-uteriene vruchtdood: zie bijlage I, tabel 1

Maturatie:
☐ normaal
☐ afgenomen
☐ versneld

Kernhoudende erythrocyten
☐ normaal in aantal
☐ in > 2 capillairen bij 10x vergroting (aantal gering verhoogd)
☐ in > 50% van de capillairen (aantal sterk verhoogd)

Infarcering:
☐ nee
☐ ja

Perivilleuze fibrine depositie
☐ < 5% van de villi omgeven door fibrine
☐ ≥ 5%-<20% van de villi omgeven door fibrine
☐ ≥ 20%-<50% van de villi omgeven door fibrine
☐ ≥ 50% van de villi omgeven door fibrine

Intervilleuze thrombose
☐ nee
☐ ja

Thrombose foetale vaten in choriaal plaat en stamvilli	☐	nee
	☐	ja
Intramurale fibrine depositie foetale vaten	☐	nee
in choriaal plaat en stamvilli	☐	ja
Focus met ≥ 5 fibrotische avasculaire	☐	nee
villi zonder ontsteking of ijzer depositie	☐	ja
Hemorrhagische endovasculitis	☐	nee
	☐	ja ☐ focaal ☐ diffuus
Vasculitis	☐	nee
	☐	ja
Subchoriale intervillositis	☐	nee
	☐	ja
Chronische villitis	☐	nee
	☐	ja, zie bijlage I, tabel 3
Acute villitis	☐	nee
	☐	ja
Chronische intervillositis	☐	nee
	☐	ja

Aanvullend onderzoek (op indicatie):

DNA flowcytometrisch onderzoek:

☐ niet verricht ☐ verricht, uitslag:
☐ diploid; ☐ triploid; ☐.....

Microbiologisch onderzoek:

☐ niet verricht ☐ verricht, uitslag:

DEFINITIEVE BEVINDINGEN PLACENTA ONDERZOEK:

Normaal	☐ nee ☐ ja	
Tijdstip intra-uteriene vruchtdood	☐ < 48 uur voor de partus	
	☐ ≥ 48 uur voor de partus	
	☐ ≥ 14 dagen voor partus	
Aantal kernhoudende erytrocyten	☐ normaal	
	☐ verhoogd	
Chorangiosis	☐ nee	
	☐ ja	
Coiling-index navelstreng	☐ normaal (1-3 windingen/10cm)	
	☐ verlaagd (≤ 1 winding/10cm)	
	☐ verhoogd (≥ 3 windingen/10cm)	
Afwijkingen passend bij placenta-bed	☐ nee	
pathologie	☐ ja	
Afwijkingen passend bij abruptio placentae	☐ nee	
	☐ ja	
Intra-uteriene infectie	☐ nee	
	☐ ja	☐ opstijgend
		☐ haematogeen
Ernstige (graad 4) chronische villitis	☐ nee	
(zie bijlage I, tabel 3)	☐ ja	
Chronische intervillositis	☐ nee	
	☐ ja	
Massale perivilleuze fibrine depositie/	☐ nee	
maternaal vloer infarct	☐ ja	
Beeld passend bij foeto-maternale bloeding	☐ nee	
(intervilleuze thrombose)	☐ ja	
Foetale thrombotische vasculopathie	☐ nee	
(zie bijlage I, tabel 4)	☐ ja	
Terminale villus deficiëntie	☐ nee	
	☐ ja	
Beeld passend bij diabetus mellitus	☐ nee	
	☐ ja	
Partiële mola	☐ nee	
	☐ ja	
Pseudo-partiële mola/ mesenchymale dysplasie	☐ nee	
	☐ ja	

CONCLUSIE:

Causaal verband obductie bevindingen, placenta onderzoek en IUVD

☐ **NEE**

☐ **JA,**

nl:

BIJLAGE I

TABEL 1. Bepaling tijdstip van intra-uterien overlijden

Obductie

Macroscopie:

☐	≥ 6 uur	navelstreng(stomp) verkleuring
☐	≥ 6 uur	epidermolysis ≥ 1cm huid oppervlak
☐	≥ 12 uur	epidermolysis gelaat, rug of buik
☐	≥ 18 uur	epidermolysis ≥ 5 % van lichaams oppervlak
☐	≥ 18 uur	epidermolysis ≥ 2 regio's van het lichaamsoppervlak (schedel, gezicht, nek, borst, buik, rug, armen, handen, benen, voeten, scrotum)
☐	≥ 24 uur	bruine verkleuring van de huid
☐	≥ 2 weken	mummificatie

Genest and Singer Obstet Gynecol 1992; 80: 593-600

Microscopie:

Verlies van kernbasofilie

☐	≥ 4 uur	renale corticale tubuli
☐	≥8 uur	mucosaal epitheel tr gastrointestinalis
☐	≥24 uur	lever, kraakbeen, binnenste deel myocard
☐	≥ 36 uur	volledig in pancreas
☐	≥ 48 uur	glomeruli, buitenste deel myocard
☐	≥ 72 uur	definitieve schors bijnieren, transmuraal tr gastrointestinalis
☐	≥ 96 uur	bronchiaal epitheel en volledig in lever
☐	≥ 1 week	kraakbeen trachea en volledig in tr gastrointestinalis en bijnieren
☐	≥ 2 weken	alveolaire septa
☐	≥ 4 weken	volledig in nieren
☐	≥ 8 weken	volledig in longen en cerebrale cortex

Genest et al Obstet Gynecol 1992; 80: 575-584

Placenta onderzoek

☐	≥ 6 uur	intravasculaire karyorrhexis
☐	≥ 48 uur	multifocale vaat afwijkingen in stamvilli
☐	≥ 14 dagen	uitgebreide vaat afwijkingen in stamvilli
☐	≥ 14 dagen	uitgebreide fibrose van terminale villi met vaat obliteratie

Genest Obstet Gynecol 1992; 80: 585-592

TABEL 2a. Maten en gewichten in relatie tot amenorrhoeduur (AD) of lichaamsgewicht.

	Gemeten	Normaal waarde in relatie tot AD	↑↓ =	Normaal waarde in relatie tot gewicht	↑↓ =
Lichaamsgewicht					
Kruin-stuitlengte					
Kruinhiellengte					
Voetlengte					
Femurlengte					
Schedelomvang					
Borstomvang					
Buikomvang					
Thymus					
Hart					
Long links					
Long rechts					
Lever					
Milt					
Bijnier links					
Bijnier rechts					
Nier links					
Nier rechts					
Hersenen					
Placenta[1]					
Placenta afmetingen[2]					
Navelstreng (NS) lengte					
Aantal NS windingen					
Coiling-index[3]					

[1]gewicht na afname van vliezen en navelstreng, [2] maximale- en minimale diameter en dikte, [3] aantal windingen/ 10cm navelstreng. Organ weights in Pediatric Pathology, second edition. Stocker and Dehner (eds). Lippincott Williams & Wikins, 2001

TABEL 2b. Ratio's gewichten.

Gemeten	↑↓ =	Normaal waarde
Long:lichaam		<28 weken AD ≥0.015:1; ≥ 28 weken AD ≥0.012:1
Lever:hersenen		1:3
Hart:lever		1:5 (1:4-1:7)
Hart:longen		1:3 (1:2-1:4)
Bijnieren:nieren		< 37 weken AD 1:2; ≥ 37 weken AD 1:4
Thymus:milt:bijnieren		1:1:1
Placenta:lichaam		afhankelijk van AD

TABEL 3. Gradering chronische villitis

☐ Graad 1, focaal:	< 5 villi/ focus in 1 coupe
☐ Graad 2, multifocaal:	< 5 villi/ focus in meerdere coupes
☐ Graad 3, patchy:	> 5 villi/ focus
☐ Graad 4, diffuus:	> 5% van de terminale villi aangedaan

Disorders of the placental parenchyma in Pathology of the placenta, second edition. Lewis and Perrin (eds). Churchill Livingstone, 1999

TABEL 4. Foetale thrombotische vasculopathie (ten minste een van deze criteria)

☐ Focus met ≥ 5 fibrotische avasculaire chorionvilli zonder ontsteking of depositie van ijzer
☐ Thrombose van foetale vaten in de navelstreng (NS), choriaalplaat (CP) of stamvilli (SV)
☐ Fibrine depositie ± calcificatie in de wand van foetale vaten in de NS, CP of SV
☐ Hemorrhagische endovasculitis bij een < 48 uur voor de partus overleden foetus

Redline and Pappin Hum Pathol 1995; 30: 80-85

Kraus and Acheen Hum Pathol 1999; 30: 759-769