

Congenital anomalies in multiple births after early loss of a conceptus

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BACKGROUND: Congenital anomalies are more common in twins than singletons but in the majority, aetiology is not known. Our aim was to test the hypothesis that survivors of an early loss in a multiple conception, compared with all singletons, are at increased risk of congenital anomaly.

METHODS: Data were abstracted from the UK population-based Northern Multiple Pregnancy Register and Northern Congenital Abnormality Survey, 1998–2004.

RESULTS: Among 3311 twin conceptions, both conceptuses were lost at <16 weeks gestation in 67, and one conceptus in 142 conceptions. Of the 142 singleton survivors, two died in infancy, two were terminated for a congenital anomaly and 11 of 138 had a congenital anomaly (prevalence 915.5 per 10 000 births). There were 197 congenital anomalies among 5948 registered twin births (331.2 per 10 000). The relative risk (RR) of congenital anomalies in a singleton with early loss of a conceptus and twins was 2.40 [95% confidence interval (CI): 1.34–4.29]. There were 4265 infants with a congenital anomaly among the 206 914 singletons [206.1 per 10 000 births: RR twin: singleton 1.61 (95% CI 1.40–1.89)].

CONCLUSIONS: A highly significant increase in the risk of congenital anomaly in survivors from a multiple conception following early loss of a conceptus supports the hypothesis that many congenital anomalies are associated with monozygotic multiple conceptions.

Key words: multiple pregnancies / twins / congenital anomalies / early fetal loss

Introduction

Fetal death is defined as death prior to the complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of pregnancy (World Health Organisation, 1993). Late fetal deaths, after 24 weeks gestation in twin gestations, are associated with major morbidity such as cerebral palsy and various congenital anomalies in the surviving co-conceptus (Melnick, 1977; Hagay *et al.*, 1986; Carlson and Towers, 1989; Cherouny *et al.*, 1989; Fusi and Gordon, 1990; Van den Veyver *et al.*, 1990; Ishimatsu *et al.*, 1994; Kilby *et al.*, 1994; Lin *et al.*, 1999; Pharoah and Adi, 2000; Weiss *et al.*, 2004). Whether or not early fetal death, before 24 weeks gestation, may be associated with similar risks of death or morbidity in the co-twin is less certain. There have been case reports of second and first trimester fetal deaths with a variety of congenital anomalies in the surviving co-twin (Saier *et al.*, 1975; Hoyme *et al.*, 1981; Baker and Doering, 1982; Anderson *et al.*, 1990; Van Bogaert *et al.*,

1996; Baker *et al.*, 1996). As these are case reports, it is not possible to ascertain whether the observed anomalies are chance observations or whether they indicate an increased risk attributable to early fetal demise of a conceptus. Population-based risks are required but such data are scarce.

It has been hypothesized that a significant proportion of infants with cerebral palsy may be attributable to very early loss of one conceptus in a twin gestation (Pharoah and Cooke, 1997). Infants with cerebral palsy are at increased risk of having a coincidental congenital anomaly, an observation that has been used to infer that the cerebral impairment presenting as cerebral palsy occurs during fetal development (Nelson and Ellenberg, 1985; Blair and Stanley, 1993; Palmer *et al.*, 1995; Croen *et al.*, 2001). The coincidence of both cerebral palsy and a congenital anomaly in an infant led to the hypothesis that a common pathogenic process was responsible for the dual impairment. It has been postulated that both congenital anomalies and cerebral palsy may be attributable to early fetal loss of a conceptus

in a multiple gestation (Pharoah, 2005). Further, it has been proposed that the initiation of the pathogenic process was division of the zygote but the subsequent pathogenic pathways for both cerebral palsy and congenital anomalies diverged (Pharoah, 2007).

Many congenital anomalies occur during very early fetal development. Thus, the effects of early fetal loss of one conceptus on the development of the surviving conceptus need to be explored. The aim of this study was to test the hypothesis that survivors of early loss in a multiple conception are at increased risk of a congenital anomaly.

Materials and Methods

The Northern Region of England is a geographically defined area with a stable population of almost 3 million and ~31 000 births per year, residing in two main urban conurbations and extended rural areas. It comprises the counties of North Cumbria, Northumberland, Tyne and Wear, Durham and Darlington and Teesside. The total number of registered births for the study period, 1998–2004, was obtained from the Office for National Statistics.

The Northern Multiple Pregnancy Register (MPR) was established in 1998 to capture details on all multiple pregnancies in the Northern Region (Glinianaia *et al.*, 2002; Ward Platt *et al.*, 2006). Ascertainment is from the earliest antenatal scan on which a multiple pregnancy is detected, and then successively at the time of the 20 week anomaly scan and at delivery. At the first trimester ultrasound screening, the number of sacs/embryos and heartbeat are detected and chorionicity, when possible, is determined. The final diagnosis of chorionicity for like-sex twin pregnancies is based on placental examination and histology or, when there is no pathological examination of the placenta, on the appropriate first trimester ultrasound. The records are linked to the Perinatal Mortality Survey (PMS) database (Northern Regional Health Authority Coordinating Group, 1984) and the Northern Congenital Abnormality Survey (NorCAS) (Richmond and Atkins, 2005). Data on congenital anomalies in fetal deaths and live births, whether diagnosed antenatally or not, are notified from multiple sources, including antenatal ultrasound, fetal medicine, cytogenetic laboratories, the regional cardiology centre and the departments of pathology and paediatric surgery to NorCAS thereby ensuring high case ascertainment.

NorCAS is a member of the British Isles Network of Congenital Anomaly Registers (BINOCAR; Rankin, 2007) and the European Surveillance of Congenital Anomalies (EUROCAT: a network of 38 congenital anomaly registers in 20 European countries). The EUROCAT exclusion list for minor congenital anomalies (patent ductus arteriosus in a premature infant, undescended testis, birthmarks, skin tags, unspecified talipes and macrocephaly) is employed by NorCAS (<http://www.eurocat.ulster.ac.uk/>). Further details on data collection have been reported previously (Richmond and Atkins, 2005).

Definitions

An early fetal death was defined as loss of a conceptus at <16 weeks gestation in a multiple conception that was diagnosed at the first ultrasound examination. A spontaneous or therapeutic abortion was defined as loss of a conceptus that occurred between ≥ 16 and <24 weeks of gestation.

A distinction is made between conceptions and registered births because spontaneous and therapeutic abortions at <24 weeks gestation are included in the figures but are not usually registered as a birth. Thus, for twin conceptions where one fetus is spontaneously or therapeutically aborted, the surviving conceptus is counted as a singleton birth.

The prevalence of congenital anomalies in twins was determined per 10 000 twin still and live births.

Congenital anomalies were coded according to the International Statistical Classification of Diseases and Related Health Problems, Chapter XVII (Q rubric) 'Congenital Malformations, Deformations and Chromosomal Abnormalities, 10th revision' (ICD-10) (World Health Organisation, 1993).

Statistics

Relative risks (RR) were determined using the statistical package EpiInfo 6 with Yates' correction.

Ethics and consent

The registers are held at the Regional Maternity Survey Office in Newcastle which is part of the North East Public Health Observatory (NEPHO). Data are processed according to NEPHO's Security and Confidentiality Policy. The NorCAS has ethics approval (04/MRE04/25) to undertake studies involving the use of its data.

Results

There were 213 087 registered live and stillbirths in the Northern Region during 1998–2004 comprising 206 914 singletons, 5948 twins, 210 triplets and 15 higher-order multiple births. Among the singletons, there were 4265 with a congenital anomaly, a prevalence of 206.1 per 10 000 births.

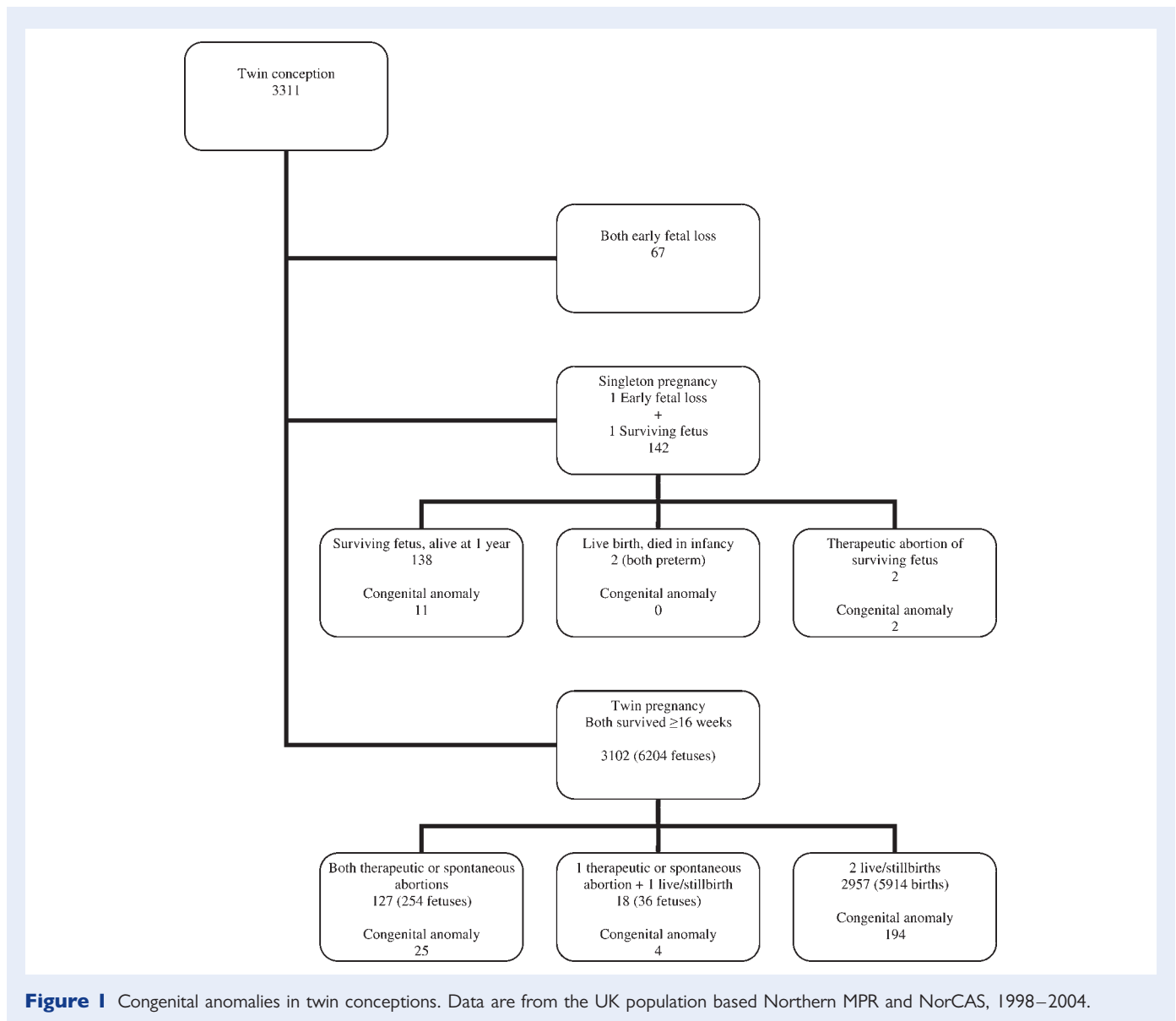
Among twin births, there were 194 congenital anomalies in 5914 births (Fig. 1) plus 3 congenital anomalies in 34 twin births from triplet conceptions (Fig. 2). Thus, there were 197 with a congenital anomaly among the 5948 registered twin births, a prevalence of 331.2 per 10 000.

The twin: singleton RR of congenital anomaly was 1.61 [95% confidence interval (CI): 1.40–1.89; $P < 0.0001$].

Among the 210 triplets born, there were eight with a congenital anomaly (prevalence 381.0 per 10 000 births).

The number of twin conceptions notified to the MPR during the 7 years was 3311. Gestational age at diagnosis was recorded in 3187 of which 2103 (66%) were diagnosed by ultrasound at <13 weeks. In 67 of these, both conceptuses suffered a late miscarriage or termination. In 142, there was loss of a conceptus at <16 weeks gestation (Fig. 1). In two, the conceptus that was lost had a congenital anomaly and in two, the surviving conceptus was therapeutically aborted for a chromosomal anomaly and 140 singleton births were registered. Two of the 140 registered singletons were neonatal deaths with prematurity as the cause of death. Eleven of the 138 that survived infancy had a congenital anomaly. The congenital anomalies in the 11 survivors of a spontaneously lost co-conceptus were: cardiac (two), skeletal (three), chromosomal (one), intestinal (two), facial clefts (one), urogenital system (one) and other (one). Thus, 13 of the 142 survivors of an early fetal loss (915.5 per 10 000 singleton survivors of early fetal loss) had a congenital anomaly.

The RR of a congenital anomaly in a singleton birth from a twin conception and twin births from a twin conception is 2.40 (95% CI: 1.34–4.29; $P < 0.01$).



A diagrammatic presentation of the outcome of the 100 triplet conceptions is shown in Fig. 2. There was early loss of all three conceptuses in 10 of these.

Spontaneous loss of one conceptus at <16 weeks gestation resulted in twin pregnancies in 14. There were three congenital anomalies among the 28 births (renal agenesis, transposition of the great vessels and ventricular septal defect), a prevalence of 1071.4 per 10 000 live births. As is the case for twin conceptions with early loss of one conceptus, the prevalence of congenital anomalies in triplet conceptions with an early loss of one conceptus was significantly different from singleton pregnancies (RR: 5.43; 95% CI: 1.86–15.82; exact $P = 0.017$).

Seventy triplet pregnancies were registered comprising 11 stillbirths, 15 infant deaths and 184 live births. Eight of the 210 registered triplet births had a congenital anomaly (381 per 10 000 births). This is of a similar order of magnitude as found for twin conceptions that result in twin registered births.

Discussion

The NorCAS belongs to established UK and European networks of congenital anomaly registers that use similar inclusion criteria and have a consistent approach to data collection, coding and recording. The use of multiple source notifications ensures NorCAS has a high case ascertainment and allows validation of the data (Richmond and Atkins, 2005). The MPR is the only active register recording multiple gestations in the UK. Multiple source ascertainment and cross-validation with the PMS, NorCAS and the Office for National Statistics contribute to its quality (Glinianaia et al., 2002). The MPR is unique in that it registers multiple gestations from the earliest antenatal scan: approximately two-thirds of twin gestations are diagnosed before 13 weeks and 90% before 19 weeks gestation (Ward Platt et al., 2006).

The majority of studies comparing the prevalence of congenital anomalies at birth in singleton and multiple births have observed an increased risk among the latter. Among twins, the higher prevalence

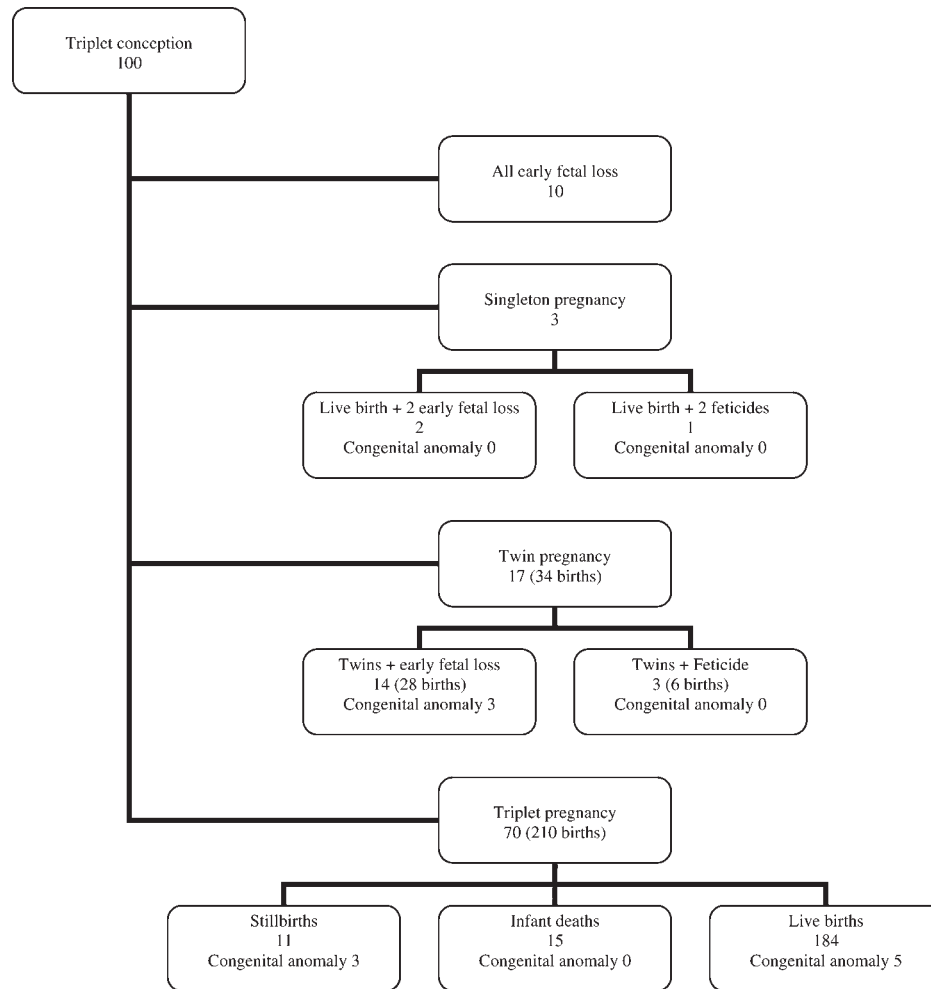


Figure 2 Congenital anomalies in triplet conception. Data are from the UK population based Northern MPR and NorCAS, 1998–2004.

of congenital anomalies occurs in monozygotic (MZ) compared with dizygotic (DZ), and in monochorionic (MC) compared with dichorionic, twins (Myriantopoulos, 1978; Cameron *et al.*, 1983; Corney *et al.*, 1983; Glinianaia *et al.*, 2008). The low concordance rate within MZ twins is a denial of the hypothesis that the majority of anomalies are the consequence of a gene segregation process (Knox and Lancashire, 1991). However, the problems of comparing prevalence at birth of congenital anomalies in singleton and multiple pregnancies arise from the bias inherent in loss of embryos or fetuses before birth. Among spontaneous abortuses, twins are about three times as frequent as live births with 88% of twin embryos and 21% of twin fetuses being abnormal (Livingstone and Poland, 1980). High loss of abnormal conceptuses in multiple gestations will lead to a serious underestimate of the prevalence of congenital anomalies in these conceptions. Furthermore, a congenital anomaly in a singleton registered birth will be falsely ascribed to a singleton conception if there has been early loss of a co-conceptus thereby inflating the congenital anomaly prevalence in singletons. Efforts to determine the true contribution of multiple gestations to the problem of congenital anomalies require that the multiplicity of all conceptions and

whether there has been early loss of a conceptus is recorded. The data recorded in the MPR and its linkage to the NorCAS and the PMS is a step towards this objective.

The data presented here confirm the well established observation that multiple births are at greater risk than singletons of having a congenital anomaly. This increase in risk is significantly enhanced if, in a multiple conception, there is an early loss of a conceptus. The risk of a congenital anomaly in the survivor following very early loss of a conceptus is over twice that of a twin pregnancy and almost 4-fold compared with a singleton pregnancy.

In addition to an inflation of the prevalence of congenital anomalies in singletons owing to twin conceptions being registered as singletons following very early loss of a co-conceptus, there is another possible source of bias in the estimation of RR. Very early ultrasound assessment of pregnancy is frequently made when monitoring pregnancies resulting from artificial reproduction therapy but current law in the UK does not permit the holding of information on the type of conception. Such pregnancies are almost invariably DZ except for the slightly greater propensity for MZ division (Derom *et al.*, 1987; Wenstrom *et al.*, 1993). Spontaneous conceptions are more likely

to undergo routine ultrasound assessment later in gestation. Such a bias will lower the estimate of risk attributable to very early loss of a conceptus in an MZ conception.

Environmental and other teratogens, nutritional deficiencies and gene segregation processes are unable satisfactorily to account for the singleton/multiple differences in congenital anomaly prevalence and other pathogenic mechanisms must be invoked. In addition to the hypothesis tested in this study, it has been hypothesized that cerebral palsy of pre-partum aetiology is also the result of early loss of a conceptus in a multiple gestation (Pharoah and Cooke, 1997) and that both cerebral palsy and congenital anomalies share the same pathological process though the pathological pathways themselves differ (Pharoah, 2005). If this is so, cerebral palsy of pre-partum origin is another congenital anomaly and the coincidence of cerebral palsy with some other congenital anomaly infant would be observed more frequently than expected by chance. Several reports confirm this (Nelson and Ellenberg, 1985; Blair and Stanley, 1993; Palmer et al., 1995; Croen et al., 2001).

This study provides strong evidence that loss of a conceptus in a multiple gestation makes a significant contribution to the aetiology of congenital anomalies. The proportion of congenital anomalies attributable to such a pathogenesis is not known. The linking of multiple pregnancy and congenital anomaly registers needs to be routine to achieve this. However, only half of all births in England and Wales contribute to a congenital anomaly register (Rankin, 2007). Cerebral palsy registers should also contribute to the linkage.

The importance of determining pathogenic mechanisms of disease is underlined by the potential for disease prevention. Prenatal diagnosis and termination of pregnancy is often an unsatisfactory solution. If MZ division with MC placentation plays a crucial role in many congenital anomalies, division of the blastomere needs to be the focus of primary prevention. MZ division appears to be a random event and is remarkably constant at ~3.5 per 1000 maternities (Little and Thompson, 1988). A possible mechanism involves the role of calcium-dependent intercellular bonding of the blastomere. A calcium chelator increases the chances of an MZ multifetal pregnancy (Steinmann, 2002). Further investigation of factors influencing MZ division may have important preventive potential.

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References

Anderson RL, Golbus MS, Curry CJR, Callen PW, Hastrup WH. Central system damage and other anomalies in surviving fetus following

- second trimester antenatal death of a co-twin. Report of four cases and literature review. *Prenat Diagn* 1990;**13**:513–518.
- Baker VV, Doering MC. Fetus papyraceus: an unreported congenital anomaly of the surviving infant. *Am J Obstet Gynecol* 1982;**143**:234–235.
- Baker EM, Khorasgani MG, Gardner-Medwin D, Gholkar A, Griffiths PD. Arthrogryposis multiplex congenital and bilateral parietal microgyria in association with the intrauterine death of a twin. *Neuropediatrics* 1996;**27**:54–56.
- Blair E, Stanley F. When can cerebral palsy be prevented? The generation of causal hypotheses by multivariate analysis of a case-control study. *Paediatr Perinat Epidemiol* 1993;**7**:272–301.
- Cameron AH, Edwards JH, Derom R, Thiery M, Boelaert R. The value of twin studies in the study of malformations. *Eur J Obstet Gynecol Reprod Biol* 1983;**14**:347–356.
- Carlson NJ, Towers CV. Multiple gestation complicated by the death of one fetus. *Obstet Gynecol* 1989;**73**:685–689.
- Cherouny PH, Hoskins IA, Johnson TRB, Niebyl JR. Multiple pregnancy with late death of one fetus. *Obstet Gynecol* 1989;**74**:318–320.
- Corney G, MacGillivray I, Campbell DM, Thompson B, Little J. Congenital anomalies in twins in Aberdeen and North-East Scotland. *Acta Genet Med Gemellol* 1983;**32**:31–35.
- Croen LA, Grether JK, Curry CJ, Nelson KB. Congenital abnormalities among children with cerebral palsy: More evidence for prenatal antecedents. *J Pediatr* 2001;**138**:804–810.
- Derom C, Derom R, Vlietinck R, Van Den Berghe H, Thiery M. Increased monozygotic twinning rates after ovulation induction. *Lancet* 1987;**i**:1236–1238.
- Fusi L, Gordon H. Twin pregnancy complicated by single intrauterine death. Problems and outcome with conservative management. *Br J Obstet Gynaecol* 1990;**97**:511–516.
- Glinianaia SV, Rankin J, Wright C, Sturgiss SN, Renwick M. A Multiple Pregnancy Register in the North of England. *Twin Res* 2002;**5**:436–439.
- Glinianaia SV, Rankin J, Wright C. Congenital anomalies in twins: a register-based study. *Hum Reprod* 2008;**23**:1306–1311.
- Hagay ZJ, Mazor M, Leiberman JR, Biale Y. Management and outcome of multiple pregnancies complicated by the antenatal death of one fetus. *J Reprod Med* 1986;**8**:717–720.
- Hoyme HE, Higginbottom MC, Jones KL. Vascular etiology of disruptive structural defects in monozygotic twins. *Pediatrics* 1981;**67**:288–291.
- Ishimatsu J, Hori D, Miyajima S, Hamada T, Yakushiji M, Nishimi T. Twin pregnancies complicated by the death of one fetus in the second or third trimester. *J Matern Fetal Invest* 1994;**4**:141–145.
- Kilby MD, Govind A, O'Brien PMS. Outcome of twin pregnancies complicated by a single intrauterine death: a comparison with viable twin pregnancies. *Obstet Gynecol* 1994;**84**:107–109.
- Knox G, Lancashire RJ. *Epidemiology of Congenital Malformations*. London: HMSO, 1991.
- Lin I-J, Chen C-H, Wang T-M, Fu L-S, Chi C-S. Infants of twin pregnancies with one twin demise in the uterus: a retrospective study. *Acta Paediatr Sin* 1999;**40**:92–96.
- Little J, Thompson B. Descriptive epidemiology. In: MacGillivray I, Campbell DM, Thompson B (eds). *Twinning and Twins*. New York: Wiley, 1988.
- Livingstone JE, Poland BJ. A study of spontaneously aborted twins. *Teratology* 1980;**21**:139–148.
- Melnick M. Brain damage in a survivor after in utero death of monozygous co-twin. *Lancet* 1977;**ii**:1287.
- Myriantopoulos MC. Congenital malformations. The contribution of twin studies. *Birth Defects* 1978;**14**:151–159.
- Nelson KB, Ellenberg JH. Antecedents of cerebral palsy. I. Univariate analysis of risks. *Am J Dis Child* 1985;**139**:1031–1038.

- Northern Regional Health Authority Coordinating Group. Perinatal mortality: a continuing collaborative regional survey. *Br Med J* 1984; **288**:1717–1720.
- Palmer L, Blair E, Petterson B, Burton P. Antenatal antecedents of moderate and severe cerebral palsy. *Paediatr Perinat Epidemiol* 1995; **9**:171–184.
- Pharoah POD. Causal hypothesis for some congenital anomalies. *Twin Res Hum Genet* 2005; **8**:543–550.
- Pharoah POD. Prevalence and pathogenesis of congenital anomalies in cerebral palsy. *Arch Dis Child Fetal Neonatal Ed* 2007; **92**:F489–F493.
- Pharoah POD, Adi Y. Consequences of in-utero death in a twin pregnancy. *Lancet* 2000; **355**:1597–1602.
- Pharoah POD, Cooke RWI. A hypothesis for the aetiology of spastic cerebral palsy—the vanishing twin. *Dev Med Child Neurol* 1997; **39**:292–296.
- Rankin J. Congenital anomalies in the British Isles. In: Nicolopoulou-Stamati P, Hens L, Howard CV (eds). *Congenital Diseases and the Environment*. Springer, 2007, 359–377.
- Richmond S, Atkins J. A population-based study of the prenatal diagnosis of congenital malformation over 16 years. *Br J Obstet Gynaecol* 2005; **112**:1349–1357.
- Saier F, Burden L, Cavanagh D. Fetus papyraceus: an unusual case with congenital anomaly of the surviving fetus. *Obstet Gynecol* 1975; **44**:217–220.
- Steinmann G. Mechanisms of twinning V: conjoined twins, stem cells, and the calcium model. *J Reprod Med* 2002; **47**:313–321.
- Van Bogaert P, Donner C, David P, Rodesch F, Avni EF, Szliwowski HB. Congenital bilateral perisylvian syndrome in a monozygotic twin with intra-uterine death of the co-twin. *Dev Med Child Neurol* 1996; **38**:166–171.
- Van den Veyver IBM, Schatteman E, Vanderheyden JS, Van Wiemeersch J, Meulyzer P. Antenatal fetal death in twin pregnancies: a dangerous condition mainly for the surviving co-twin: a report of four cases. *Eur J Obstet Gynecol* 1990; **38**:69–73.
- Ward Platt M, Glinianaia SV, Rankin J, Wright C, Renwick M. The North of England Multiple Pregnancy Register: five year results of data collection. *Twin Res Hum Genet* 2006; **9**:913–918.
- Weiss JL, Cleary-Goldman J, Tanji K, Budorick N, D'Alton ME. Multicystic encephalomalacia after first trimester death in monochorionic twins. *Am J Obstet Gynecol* 2004; **190**:563–565.
- Wenstrom KD, Syrop CH, Hammitt EG, Van Voorhis BJ. Increased risk of monochorionic twinning associated with assisted reproduction. *Fertil Steril* 1993; **60**:510–513.
- World Health Organisation. *International Statistical Classification of Disease and Related Health Problems*, 10th revision. Geneva: WHO, 1993.

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