

Gender mix in twins and fetal growth, length of gestation and adult cancer risk

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Summary

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This study evaluated the effect of gender mix (the gender combinations of twin pairs) on fetal growth and length of gestation, and reviewed the literature on the long-term effects of this altered fetal milieu on cancer risk. In singletons, it is well established that females weigh less than males at all gestations, averaging 125–135 g less at full term. This gender difference is generally believed to be the result of the effect of androgens on fetal growth. The gender difference in fetal growth is greater before the third trimester and less towards term, with males growing not only more, but also earlier than females. Plurality is a known risk factor for reduced fetal growth and birthweight. Compared with singletons, the mean birthweight percentiles of twins fall substantially (by 10% or more) below the singleton 10th percentile by 28 weeks, below the singleton 50th percentile by 30 weeks, and below the singleton 90th percentile by 34 weeks. In unlike-gender twin pairs, it has been reported that the female prolongs gestation for her brother, resulting in a higher birthweight for the male twin than that of like-gender male twins. Other researchers have demonstrated that females in unlike-gender pairs had higher birthweights than females in like-gender pairs.

Analyses from our consortium on 2491 twin pregnancies with known chorionicity showed longer gestations and faster rates of fetal growth in both males and females in unlike-gender pairs compared with like-gender male or female pairs, although these differences were not statistically significant. The post-natal effects for females growing in an androgenic-anabolic environment include increased sensation-seeking behaviour and aggression, lowered visual acuity, more masculine attitudes and masculinising effects of the auditory system and craniofacial growth. In contrast, there is no evidence to suggest that there might be a similar feminising effect on males from unlike-gender pairs. This hormonal exposure *in utero* may influence adult body size and susceptibility to breast cancer.

Introduction

Twin pregnancies provide the unique opportunity to study the influence of an additional fetus on intrauterine growth and pregnancy outcome, as well as any long-lasting effects influencing adult health and dis-

ease risk. In singletons, these associations between the fetal environment and adult health have been the topic of international debate and research interest as the fetal origins of adult disease.^{1–5} In this paper we will examine the intrauterine effects of gender mix (the gender

combinations of twin pairs) on fetal growth and length of gestation, and present a review of the literature evaluating the long-term effects of this altered fetal milieu on cancer risk.

Singletons

It is well established that female infants weigh less than male ones at all gestational ages, yet are more likely to survive.^{6,7} Even among extremely low birthweight infants (<1000 g), female gender is associated with a better than threefold odds of survival (adjusted odds ratio [AOR] 3.06, 95% confidence interval [CI] 1.71, 5.45).⁸ Among full-term singletons, white male infants average 135 g and black male infants average 125 g heavier than their respective female counterparts.⁹ The gender difference is generally believed to be the result of androgens on fetal growth. This is demonstrated in male newborns with hypospadias and reduced levels of androgens.¹⁰ Data from the Avon Longitudinal Study of Parents and Children showed that, after adjusting for gestation, males with hypospadias had significantly reduced overall growth compared with healthy males, averaging 226 g lighter in birthweight, 0.8 cm shorter in birth length, and 0.6 cm smaller in head circumference.

The action of androgens in males increases not only the rate of fetal weight gain, but may also alter its time course *in utero*.^{11,12} The gestational age at which children are born with a given weight varies by gender, with males born significantly and consistently younger than females; the age difference is most pronounced at lower birthweights. The gender difference in fetal growth is greater before the third trimester and less towards term, with males growing not only more, but also earlier than females. Pedersen¹³ reported significant first trimester gender differences in growth by ultrasound. Males averaged 2.0 mm longer crown-rump length by 8–12 weeks of gestation and 1.4 mm larger biparietal diameter by 16 weeks of gestation than females, resulting in 40 g heavier birthweight and 4 mm longer birth length. These gender differences in fetal size increased with advancing gestation, from approximately 1 day at 8–12 weeks of gestation to 6–7 days at term. It is speculated that some critical time windows of development may be slightly different between males and females, and that this phenomenon may be one of the reasons for gender differences in sensitivity to fetal programming. For example, reduced abdominal circumference at birth is positively

associated with plasma concentrations of fibrinogen,¹⁴ serum cholesterol and serum apolipoprotein B in adult life,¹⁵ as well as death from coronary heart disease;¹⁶ all associations are stronger in men than in women, and are independent of length of gestation. It has been shown in animal models that the fetal pituitary gland regulates the onset of labour. In humans, this role has been supported by delayed onset of labour in fetal anencephaly.¹⁷ The fetal secretion of stress hormones is one factor precipitating labour.¹⁸ There appears to be a U-shaped relationship regarding male gender and length of gestation, with studies reporting an excess of males in preterm^{19–23} and post-term births.²⁴

Several studies have reported significantly higher maternal serum human chorionic gonadotropin levels in uncomplicated pregnancies with a female vs. a male fetus.^{25–27} In pre-eclamptic vs. normotensive mothers, those pregnant with males had significantly higher levels of both human chorionic gonadotropin and testosterone serum levels, whereas in mothers pregnant with females only testosterone levels were elevated,²⁸ indicating an androgen influence on the pathophysiological mechanism of pre-eclampsia.

Twins

Plurality is a known risk factor for reduced birthweight. Cogswell and Yip⁹ report that the difference in mean birthweight between full-term singleton and twin births is about 690 g among white infants and 630 g among black infants; the difference in mean birthweight between twin and triplet births is >500 g for both white and black infants. Min *et al.*²⁹ have demonstrated that, although twins generally grow more slowly than singletons, the overall pattern of fetal growth for well-grown twins does not differ as much as from singletons as previously believed. Compared with singletons,³⁰ the mean birthweight percentiles of twins fall substantially (by 10% or more) below the singleton 10th percentile by 28 weeks, below the singleton 50th percentile by 30 weeks and below the singleton 90th percentile by 34 weeks.²⁹ Glinianaia *et al.*³¹ also reported that twins and singletons have similar birthweight curves before 30 weeks, with progressive divergence with advancing gestation. Smith *et al.*³² reported that the fetal growth velocity for biparietal diameter, abdominal circumference and femur length in twins decreased with gestation advancing, with the downward trend more noticeable after 32 weeks; maximum and minimum growth occurred at 22–23 weeks

and 36–37 weeks respectively, unrelated to birth order within the twin pair, fetal gender, chorionicity or zygosity.

Gender mix

Surprisingly few twin studies have considered the combined effect of both siblings in the pair on length of gestation and birthweight.^{22,23,33–37} Cooperstock *et al.*²³ reported a 9.2% excess of male fetuses in white twin births before 35 weeks of gestation, based on the matched file of Missouri twin births from 1978–90. James^{38,39} suggests that males are born earlier than females because they are conceived earlier. He also suggests that, assuming equal availability of nutrients in dichorionic twin pregnancies, the males would be more successful in their competition for nutrients than their female siblings because females are programmed to grow more slowly, compared with males in like-gender twin pregnancies.³⁹ Loos *et al.*^{33,34} examined the effect of gender mix in dizygotic twin pregnancies from the East Flanders Prospective Twin Survey on birthweight and length of gestation. They reported that length of gestation in unlike-gender pairs was similar to that of female like-gender pairs, and significantly (0.4 weeks; $P = 0.02$) longer than that of male like-gender pairs. Birthweight of girls from unlike-gender pairs was similar to that of girls from like-gender pairs, but boys from unlike-gender pairs weighed 78 g more than boys from like-gender pairs ($P = 0.001$). These researchers concluded that in unlike-gender pairs it is the girl that prolongs gestation for her brother, resulting in a higher birthweight than that of like-gender boys.

Glinianaia *et al.*³⁵ evaluated the effect of gender mix on birthweight using the New Norwegian Twin Panel, which included all twin births from 1967–74 and still living in Norway in 1992. They showed that although they had comparable length of gestation, females in unlike-gender pairs had higher birthweights (by 37.6 g) than females in like-gender pairs. Birth order within the twin pair also had a significant effect, with first-born females being 68 g heavier than second-born females in unlike-gender pairs, and 55 g heavier in like-gender pairs. Blumrosen *et al.*³⁶ using data from the Israeli Birth Registry (which did not include gestational age), reported significantly higher birthweights of females, but not males, from like- vs. unlike-gender pairs. Orlebeke *et al.*³⁷ reported that birthweight was not affected by the gender of the co-twin, although

boys averaged 89 g heavier than girls and first-born twins averaged 61 g heavier than second-born twins.

Data from our consortium on 2491 twin pregnancies with known chorionicity are presented in Table 1. Among dichorionic twin pregnancies, unlike-gender pairs had a longer gestation by about 2.1 days ($P = 0.08$) compared with like-gender boys and 1.4 days (NS) compared with like-gender girls. Boys in unlike-gender pairs were growing faster than boys in like-gender pairs (+2.3 g/week between 20 and 28 weeks and +1.8 g/week between 28 weeks and delivery, NS). Girls in unlike-gender pairs were growing faster in mid-gestation (+4.6 g/week between 20 and 28 weeks, NS), but slower in late gestation (–4.2 g/week from 28 weeks to delivery, NS). As a consequence, birthweight (adjusted for gestation) was only 20 g (NS) heavier for boys and 6 g (NS) heavier for girls in unlike-gender vs. like-gender pairs. These findings are in line with those of Glinianaia *et al.*³⁵ of small increases in mean birthweights in unlike-gender twins compared with their like-gender counterparts. Also in agreement with the findings of Loos *et al.*,^{33,34} we found the length of gestation to be 0.3 weeks longer for unlike-gender pairs than for like-gender boys, but in contrast, we found it also to be 0.2 weeks longer than like-gender girls, whereas Loos *et al.* reported 0.3 weeks shorter. This difference may in part be explained by our grouping by chorionicity and gender and by the ethnic diversity of our twins, whereas Loos *et al.*^{33,34} used zygosity and gender, and their sample was ethnically homogenous. In evaluating the effects of chorionicity in like-gender twin pairs, we found length of gestation to be longer in dichorionic vs. monochorionic pregnancies, averaging about 4.9 days for girls ($P = 0.004$) and 1.4 days for boys (NS), with heavier average birthweights (102 g in girls, NS, and 171 g in boys, $P = 0.006$) (Table 1). Fetal growth rates were not significantly different among like-gender twins by chorionicity.

Twin pregnancies are at higher risk for pre-eclampsia compared with singletons, and male gender has been implicated as being a possible contributing factor. Basso and Olsen,⁴⁰ in their analysis of the Danish National Birth Registry and Statistics Denmark's Fertility Database of births from 1980–96, reported an excess of males in pregnancies complicated by pre-eclampsia. Among twin pregnancies compared with singletons, the overall odds ratio (OR) for pre-eclampsia was 2.49 [95% CI 2.25, 2.75]; by gender mix it was 2.23 [1.89, 2.68] for unlike-gender pregnancies,

Table 1. Effect of gender mix and chorionicity on rates of fetal growth, length of gestation, and birthweight in twins^a

		Gender mix of twin pairs			P-value		
		G-G	G-B	B-B	G-G vs. G-B	G-G vs. B-B	G-B vs. B-B
(N, pairs)	Di ^b	481	959	551			
	Mono ^b	250		250			
Length of gestation (weeks)	Di	35.9 ± 0.1	36.0 ± 0.1	35.7 ± 0.1	0.31	0.55	0.08
	Mono	35.2 ± 0.2		35.5 ± 0.2			
Mono vs. Di P-value ^c		0.004		0.23			
Birthweight (g)							
Females	Di	2311 ± 19	2317 ± 13		0.81		
Males	Di		2430 ± 13	2410 ± 18			0.36
Average	Di	2302 ± 17	2369 ± 12	2410 ± 16	0.001	<0.0001	0.04
Average	Mono	2200 ± 23		2239 ± 23			
Mono vs. Di P-value		0.65		0.006			
Rates of fetal growth (g/week)							
Early (0–20 weeks)							
Female	Di	16.8 ± 0.6	16.6 ± 0.4		0.79		
Male	Di		16.9 ± 0.4	17.4 ± 0.5			0.44
Average	Di	16.7 ± 0.5	16.9 ± 0.4	17.4 ± 0.5	0.69	0.30	0.41
Average	Mono	15.7 ± 0.6		15.9 ± 0.6			
Mono vs. Di P-value		0.12		0.14			
Mid (20–28 weeks)							
Female	Di	81.3 ± 3.1	85.9 ± 2.4		0.29		
Male	Di		89.6 ± 2.4	87.3 ± 3.1			0.56
Average	Di	81.5 ± 3.1	87.4 ± 2.4	87.3 ± 3.0	0.13	0.18	0.99
Average	Mono	81.3 ± 3.5		94.3 ± 4.2			
Mono vs. Di P-value		0.30		0.34			
Late (28 weeks–birth)							
Female	Di	141.1 ± 3.4	136.9 ± 2.1		0.29		
Male	Di		144.1 ± 2.1	142.3 ± 2.8			0.60
Average	Di	141.2 ± 3.0	140.4 ± 1.9	142.1 ± 2.5	0.80	0.82	0.56
Average	Mono	132.7 ± 2.6		142.1 ± 3.4			
Mono vs. Di P-value		0.36		0.28			

G-G, girl-girl; G-B, girl-boy; B-B, boy-boy.

^aLeast square means from models adjusting for mother's age, pregravid BMI, height, black ethnicity, primiparity, smoking, pre-eclampsia, gestational diabetes, fetal reduction; also adjusting for gestation for birthweight and rates of fetal growth models.

^bDi is dichorionic placentation; mono is monochorionic placentation.

^cWithin columns significance is between average of like-gender twins, comparing monochorionic vs. dichorionic.

2.47 [2.09, 2.92] for female like-gender pregnancies, and 2.53 [2.14, 2.99] for male like-gender pregnancies. Luke *et al.*⁴¹ reported that fetal growth is reduced in twin pregnancies complicated by pre-eclampsia, an effect that precedes overt clinical symptoms in the mother. Average fetal growth of the twin pair is reduced by about 3.6 g/week between 20 and 28 weeks of gestation and by 5.7 g/week from 28 weeks to delivery.

Postnatal effects among unlike-gender twins

A growing body of literature suggests that females growing in an androgenic-anabolic fetal environment

exhibit long-lasting effects. Findings consistent with possible hormonal influences in unlike-gender pairs include increased sensation-seeking behaviour and aggression,^{42,43} lowered visual acuity,⁴⁴ more masculine attitudes,⁴⁵ masculinising effect on the female auditory system,⁴⁶ and masculinising effect on the female craniofacial growth.⁴⁷ There is no evidence to suggest that there might be a similar feminising effect on males from unlike-gender pairs; studies have reported no increased risk of breast cancer in the male of unlike-gender twins.⁴⁸ Glinianaia *et al.*³⁵ suggests that the ability of androgens and oestrogens to transfer between fetuses may differ or that female tissues may have a

greater sensitivity to a higher testosterone level *in utero*. Others⁴⁵ have hypothesised that testosterone is the hormone most likely to both transfer between fetuses and to affect fetal development. Hormonal exposure *in utero* may influence adult female bodysize⁴⁹ and susceptibility to breast cancer.⁵⁰

Cancer in twins: effect of prenatal environment

Oestrogens and other hormonal factors known to influence breast cancer risk in the adult may also play a critical role during the intrauterine period.⁵¹ Pre-eclampsia is associated with lowered levels of oestrogen and a decreased subsequent risk of breast cancer in the offspring,^{52–54} while early preterm birth, neonatal jaundice, and dizygotic twinning are associated with elevated levels and higher breast cancer risks. Early preterm birth is associated with elevated maternal and fetal levels of oestrogen.^{55,56} The subsequent risk for breast cancer for women born <33 weeks of gestation has been shown to be elevated (OR 3.96 [95% CI 1.45, 10.81]).⁵³ Pregnancy steroid hormone levels have been shown to be risk factors for breast cancer.⁵⁷ In contrast, the association between birthweight and breast cancer risk is not entirely consistent.^{54,58,59} Twin pregnancies are associated with higher levels of oestrogen than singleton pregnancies, a difference that is even more pronounced in dizygotic vs. monozygotic twin pregnancies.^{60–62} Human chorionic gonadotropin in both maternal and cord blood is higher in girl-girl twins and girl-boy twins than in boy-boy twins at delivery.²⁵

Most^{48,50,53,63,64} but not all⁶⁰ studies of cancer risk among dizygotic twins show an increased risk of breast cancer. Among female twins with male co-twins, high birthweight constitutes a strong independent risk factor for breast cancer. Kaijser *et al.*⁵⁰ reported an AOR of 2.3 [95% CI 1.09, 4.8] for each kilogram increase in birthweight for women with male co-twins, increasing to 4.3 [95% CI 1.24, 14.7] for breast cancers diagnosed before age 50. Swerdlow *et al.*⁶⁵ pooled data from four case-control studies of female twins diagnosed with breast cancer conducted in Denmark, England, Wales, Finland and Sweden. Compared with their twin sisters, the risk of breast cancer was increased for women who were thinner (OR 1.44, [95% CI 1.08, 1.91]) or taller (OR 1.27, [95% CI 0.95, 1.70]) at age 10; for women who developed breasts earlier (OR 1.53, [95% CI 1.14, 2.06]), and for women with a smaller waist-to-hip ratio at age 20 (OR 1.79, [95% CI 1.00, 3.21]). These researchers concluded that childhood growth before puberty may

affect the risk of premenopausal breast cancer, at least in women without a family history of breast cancer, and that the distribution of body fat in young adulthood may also be related to breast cancer risk. Androgens interact with oestrogens through competitive binding to sex hormone-binding globulin, and female twins with male co-twins and high birthweights are exposed *in utero* to both androgens from their brothers and to high endogenous oestrogen levels.^{61,62,66}

Verkasalo *et al.*,⁶⁷ using the nationwide record linkage of the Finnish Twin Cohort Study, the Finnish Cancer Registry and the Central Population Register, followed up 12 941 like-gender twin pairs. They concluded that the overall cancer incidence among twins resembled that among the general population, and that monozygotic co-twins of affected twins were at 50% higher risk than dizygotic co-twins. They concluded that inherited genetic factors accounted for about 18% of the liability in inter-individual variation in the risk of overall cancer, while non-genetic factors shared by twins accounted for 7% and unique environmental factors for 75%. Hemminki and Li,⁶⁸ using the nationwide Swedish Family Cancer Database on 10.2 million individuals and 62 574 twins, reported that the overall risk of cancer in like-gender or unlike-gender twins was at the level of risk for singletons. Testicular cancer was increased among like-gender twins and all twins to a standardised incidence ratio (SIR) of 1.43; the SIR of breast cancer was 1.01 and 1.04 in like-gender and unlike-gender twins respectively. Probandwise (within-pair) analysis showed increased risks for Hodgkin's disease in males and breast cancer and childhood acute lymphoid leukaemia among females.

Conclusions

Male gender is associated with a faster rate of fetal growth, shorter length of gestation and higher birthweight, resulting primarily from anabolic effect of androgens. In twins, the effect of an unlike-gender twin appears to prolong gestation for the male and increase birthweight for the female. Male fetuses are also associated with significantly higher maternal levels of human chorionic gonadotropin and testosterone levels, as well as an excess of pre-eclampsia, and both early preterm and post-term births. While many of these differences may be of little clinical significance at birth, they may have implications for future health. Although many of the studies cited are based on small sample sizes, and their reported results await confirmation from subse-

quent larger studies, it appears that the altered hormonal environment *in utero* may have important long-lasting effects on the female of a mixed gender twin pair, including higher risks for cancer in adult life.

References

- Paneth N, Susser M. Early origin of coronary heart disease (the 'Barker Hypothesis'). *British Medical Journal* 1995; **310**:411–412.
- Barker DJP. The fetal origins of coronary heart disease. *Acta Paediatrica Supplement* 1997; **422**:78–82.
- Sayer AA, Cooper C, Barker DJP. Is lifespan determined in utero? *Archives of Disease in Childhood* 1997; **77**:F161–F162.
- Eriksson JG, Forsén T, Tuomilehto J, Winter PD, Osmond C, Barker DJP. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. *British Medical Journal* 1999; **318**:427–431.
- Waterland RA, Garza C. Potential mechanisms of metabolic imprinting that lead to chronic disease. *American Journal of Clinical Nutrition* 1999; **69**:179–197.
- Chase HC. Infant mortality and weight at birth: 1960 United States birth cohort. *American Journal of Public Health* 1969; **59**:1618–1628.
- Williams RL, Creasy RK, Cunningham GC, Hawes WE, Norris FD, Tashiro M. Fetal growth and perinatal viability in California. *Obstetrics and Gynecology* 1982; **59**:624–632.
- Bottoms SF, Paul RH, Mercer BM, MacPherson CA, Caritis SN, Moawad AH, et al. Obstetric determinants of neonatal survival: antenatal predictors of neonatal survival and morbidity in extremely low birth weight infants. *American Journal of Obstetrics and Gynecology* 1999; **180**:665–669.
- Cogswell ME, Yip R. The influence of fetal and maternal factors on the distribution of birthweight. *Seminars in Perinatology* 1995; **19**:222–240.
- Hughes IA, Northstone K, Golding J, the ALSPAC Study Team. Reduced birth weight in boys with hypospadias: an index of androgen dysfunction? *Archives of Disease in Childhood. Fetal and Neonatal Edition* 2002; **87**:F150–F151.
- de Zegher F, Devlieger H, Eeckels R. Fetal growth: boys before girls. *Hormone Research* 1999; **51**:258–259.
- de Zegher F, Francois I, Boehmer ALM, Saggese G, Muller J, Hiort O, et al. Androgens and fetal growth. *Hormone Research* 1998; **50**:243–244.
- Pedersen JF. Ultrasound evidence of sexual difference in fetal size in first trimester. *British Medical Journal* 1980; **281**:1253.
- Martyn CN, Meade TW, Stirling Y, Barker DJP. Plasma concentrations of fibrinogen and factor VII in adult life and their relation to intra-uterine growth. *British Journal of Haematology* 1995; **89**:142–146.
- Barker DJP, Martyn CN, Osmond C, Hales CN, Fall CHD. Growth in utero and serum cholesterol concentrations in adult life. *British Medical Journal* 1993; **307**:1524–1527.
- Barker DJP, Martyn CN, Osmond C, Wield GA. Abnormal liver growth in utero and death from coronary heart disease. *British Medical Journal* 1995; **310**:703–704.
- Myers DA, Nathanielsz PW. Biologic basis of term and preterm labor. *Clinics in Perinatology* 1993; **20**:9–28.
- Challis J, Sloboda D, Matthews S, Holloway A, Alfaidy N, Howe D, et al. Fetal hypothalamic-pituitary adrenal (HPA) development and activation as a determinant of the timing of birth, and of postnatal disease. *Endocrine Research* 2000; **26**:489–504.
- Wen SW, Goldenberg RL, Cutter GR, Hoffman HJ, Cliver SP. Intrauterine growth retardation and preterm delivery: prenatal risk factors in an indigent population. *American Journal of Obstetrics and Gynecology* 1990; **162**:213–218.
- McGregor JA, Leff M, Orleans M, Baron A. Fetal gender differences in preterm birth: findings in a North American cohort. *American Journal of Perinatology* 1992; **9**:43–48.
- Harlow BL, Frigoletto FD, Cramer DW, Evans JK, LeFevre ML, Bain RP, et al. Determinants of preterm delivery in low-risk pregnancies: the RADIUS study group. *Journal of Clinical Epidemiology* 1996; **49**:441–448.
- Cooperstock M, Campbell J. Excess males in preterm birth: interactions with gestational age, race, and multiple birth. *Obstetrics and Gynecology* 1996; **88**:189–193.
- Cooperstock MS, Bakewell J, Herman A, Schramm WF. Effects of fetal sex and race on risk of very preterm birth in twins. *American Journal of Obstetrics and Gynecology* 1998; **179**:762–765.
- Divon MY, Ferber A, Nisell H, Westgren M. Male gender predisposes to prolongation of pregnancy. *American Journal of Obstetrics and Gynecology* 2002; **187**:1081–1083.
- Steier JA, Myking OL, Ulstein M. Human chorionic gonadotropin in cord blood and peripheral maternal blood in singleton and twin pregnancies at delivery. *Acta Obstetrica et Gynecologica Scandinavica* 1989; **68**:689–692.
- Steier JA, Myking OL, Bergsjø PB. Correlation between fetal sex and human chorionic gonadotropin in peripheral maternal blood and amniotic fluid in second and third trimester normal pregnancies. *Acta Obstetrica et Gynecologica Scandinavica* 1999; **78**:363–371.
- Danzer H, Braunstein GD, Rasor J, Forsythe AW. Maternal serum chorionic gonadotropin concentrations and fetal sex prediction. *Fertility and Sterility* 1984; **34**:336–340.
- Steier JA, Ulstein M, Myking OL. Human chorionic gonadotropin and testosterone in normal and preeclamptic pregnancies in relation to fetal sex. *Obstetrics and Gynecology* 2002; **100**:552–556.
- Min S-J, Luke B, Gillespie B, Min L, Newman RB, Mauldin JG, et al. Birth weight references for twins. *American Journal of Obstetrics and Gynecology* 2000; **182**:1250–1257.
- Hadlock FP, Harrist RB, Martinez-Poyer J. In utero analysis of fetal growth: a sonographic weight standard. *Radiology* 1991; **181**:129–133.
- Glinianaia SV, Skjærven R, Magnus P. Birthweight percentiles by gestational age in multiple births. *Acta Obstetrica et Gynecologica Scandinavica* 2000; **79**:450–458.
- Smith APM, Ong S, Smith NCS, Campbell D. A prospective longitudinal study of growth velocity in twin pregnancy. *Ultrasound in Obstetrics and Gynecology* 2001; **18**:485–487.
- Loos RJF, Derom C, Eeckels R, Derom R, Vlietinck R. Length of gestation and birthweight in dizygotic twins. *Lancet* 2001; **358**:560–561.
- Loos RJF, Derom C, Eeckels R, Derom R, Vlietinck R. Gestation and birthweight in dizygotic twins. *Lancet* 2002; **359**:171–172.

- 35 Glinianaia SV, Magnus P, Harris JR, Tambs K. Is there a consequence for fetal growth of having an unlike-sexed cohabitant in utero? *International Journal of Epidemiology* 1998; **27**:657–659.
- 36 Blumrosen E, Goldman RD, Blickstein I. Growth discordance and the effect of a male twin on birth weight of its female co-twin: a population-based study. *Journal of Perinatal Medicine* 2002; **30**:510–513.
- 37 Orlebeke JF, van Baal CM, Boomsma DI, Neeleman D. Birth weight in opposite sex twins as compared to same sex dizygotic twins. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 1993; **50**:95–98.
- 38 James WH. Why are boys more likely to be preterm than girls? Plus other related conundrums in human reproduction. *Human Reproduction* 2000; **15**:2108–2111.
- 39 James WH. Gestation and birthweight in dizygotic twins. *Lancet* 2002; **359**:171–172.
- 40 Basso O, Olsen J. Sex ratio and twinning in women with hyperemesis or pre-eclampsia. *Epidemiology* 2001; **12**:747–749.
- 41 Luke B, Min S-J, Gillespie B, Avni M, Witter FR, Newman RB, *et al.* The importance of early weight gain in the intrauterine growth and birth weight of twins. *American Journal of Obstetrics and Gynecology* 1998; **179**:1155–1161.
- 42 Resnick SM, Gottesman II, McGue M. Sensation seeking in opposite-sex twins: an effect of prenatal hormones? *Behavior Genetics* 1993; **23**:323–329.
- 43 Vierikko E, Pulkkinen L, Kaprio J, Viken R, Rose RJ. Sex differences in genetic and environmental effects on aggression. *Aggressive Behavior* 2003; **29**:55–68.
- 44 Miller EM. Reported myopia in opposite sex twins: a hormonal hypothesis. *Optometry and Visual Sciences* 1995; **72**:34–36.
- 45 Miller EM, Martin N. Analysis of the effect of hormones on opposite-sex twin attitudes. *Acta Geneticae Medicae et Gemellologiae* 1995; **44**:41–52.
- 46 McFadden D. A masculinizing effect on the auditory systems of human females having male co-twins. *Proceedings of the National Academy of Sciences USA* 1993; **90**:11900–11904.
- 47 Boklage CE. Interactions between opposite-sex dizygotic fetuses and the assumptions of Weinberg difference method epidemiology. *American Journal of Human Genetics* 1985; **37**:591–605.
- 48 Cerhan JR, Kushi LH, Olson JE, Rich SS, Zheng W, Folsom AR, *et al.* Twinship and risk of postmenopausal breast cancer. *Journal of the National Cancer Institute* 2000; **92**:261–265.
- 49 Pietiläinen KH, Kaprio J, Räsänen M, Winter T, Rissanen A, Rose RJ. Tracking of body size from birth to late adolescence: contributions of birth length, birth weight, duration of gestation, parent's size, and twinship. *American Journal of Epidemiology* 2001; **154**:21–29.
- 50 Kaijser M, Lichtenstein P, Granath F, Erlandsson G, Cnattingius S, Ekbom A. In utero exposures and breast cancer: a study of opposite-sexed twins. *Journal of the National Cancer Institute* 2001; **93**:60–62.
- 51 Trichopoulos D. Hypothesis: does breast cancer originate in utero? *Lancet* 1990; **335**:939–940.
- 52 Sanderson M, Williams MA, Daling JR, Holt VL, Malone KE, Self SG, *et al.* Maternal factors and breast cancer risk among young women. *Paediatric and Perinatal Epidemiology* 1998; **12**:397–407.
- 53 Ekbom A, Hsieh CC, Lipworth L, Adami HO, Trichopoulos D. Intrauterine environment and breast cancer risk in women: a population-based study. *Journal of the National Cancer Institute* 1997; **89**:71–76.
- 54 Ekbom A, Trichopoulos D, Adami HO, Hsieh CC, Lan SJ. Evidence of prenatal influences on breast cancer risk. *Lancet* 1992; **340**:1015–1018.
- 55 Sedin G, Bergquist C, Lindgren PG. Ovarian hyperstimulation in preterm infants. *Pediatric Research* 1985; **19**:548–552.
- 56 Mazor M, HersHKovitz R, Chaim W, Levy J, Sharony Y, Leiberman JR, *et al.* Human preterm birth is associated with systemic and local changes in progesterone/17 β -estradiol ratios. *American Journal of Obstetrics and Gynecology* 1994; **171**:231–236.
- 57 Peck JD, Hulka BS, Poole C, Savitz DA, Baird D, Richardson BE. Steroid hormone levels during pregnancy and incidence of maternal breast cancer. *Cancer Epidemiology, Biomarkers and Prevention* 2002; **11**:361–368.
- 58 Michels KB, Trichopoulos D, Robins JM, Rosner BA, Manson JE, Hunter DJ, *et al.* Birthweight as a risk factor for breast cancer. *Lancet* 1996; **348**:1542–1546.
- 59 Sanderson M, Williams MA, Malone KE, Stanford JL, Emanuel I, White E, *et al.* Perinatal factors and risk of breast cancer. *Epidemiology* 1996; **7**:34–37.
- 60 Kappel B, Hansen K, Moller J, Faaborg-Anderson J. Human placental lactogen and dU-estrogen levels in normal twin pregnancies. *Acta Geneticae Medicae et Gemellologiae* 1985; **34**:59–65.
- 61 Duff DB, Brown JB. Urinary oestriol excretion in twin pregnancies. *Journal of Obstetrics and Gynaecology of the British Commonwealth* 1974; **81**:695–700.
- 62 TambyRaja RL, Ratnam SS. Plasma steroid changes in twin pregnancies. *Progress in Clinical Biology and Research* 1981; **69A**:189–195.
- 63 Braun MM, Ahlbom A, Floderus B, Britton LA, Hoover RN. Effect of twinship on incidence of cancer of the testis, breast, and other sites (Sweden). *Cancer Causes and Control* 1995; **6**:519–524.
- 64 Hsieh CC, Lan SJ, Ekbom A, Petridou E, Adami HO, Trichopoulos D. Twin membership and breast cancer risk. *American Journal of Epidemiology* 1992; **136**:1321–1326.
- 65 Swerdlow AJ, De Stavola BL, Floderus B, Holm NV, Kaprio J, Verkasalo PK, *et al.* Risk factors for breast cancer at young ages in twins: an international population-based study. *Journal of the National Cancer Institute* 2002; **94**:1238–1245.
- 66 Hardy MJ, Humeida AK, Bahijri SM, Basalamah AH. Late third trimester unconjugated serum oestriol levels in normal and hypertensive pregnancy: relation to birthweight. *British Journal of Obstetrics and Gynaecology* 1981; **88**:976–982.
- 67 Verkasalo PK, Kaprio J, Koskenvuo M, Pukkala E. Genetic predisposition, environment and cancer incidence: a nationwide twin study in Finland, 1976–1995. *International Journal of Cancer* 1999; **83**:743–749.
- 68 Hemminki K, Li X. Cancer risks in twins: results from the Swedish Family-Cancer Database. *International Journal of Cancer* 2002; **99**:873–878.