Human Reproduction Update, Vol.14, No.1 pp. 37–47, 2008 doi:10.1093/humupd/dmm036
Advance Access publication November 16, 2007

Dizygotic twinning

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The tendency to conceive spontaneous dizygotic (DZ) twins is a complex trait with important contributions from both environmental factors and genetic disposition. Twins are relatively common and occur on average 13 times per 1000 maternities, though the twinning frequency varies over time and geographic location. This variation is mostly attributed to the differences in DZ twinning rate, since the monozygotic twinning rate is relatively constant. DZ twinning is in part under genetic control, with mothers of DZ twins reporting significantly more female family members with DZ twins than mothers of monozygotic twins. Maternal factors such as genetic history, advanced age and increased parity are known to increase the risk of DZ twins. Recent research confirmed that taller mothers and mothers with a high body mass index (30>) are at greater risk of DZ twinning. Seasonality, smoking, oral contraceptive use and folic acid show less convincing associations with twinning. Genetic analysis is beginning to identify genes contributing to the variation in twinning. Mutations in one of these genes (growth differentiation factor 9) are significantly more frequent in mothers of DZ twins. However, the mutations are rare and only account for a small part of the genetic contribution for twinning.

Keywords: twinning frequency; dizygotic twinning; GDF9; genetic factors; environmental factors

Introduction

Of the 30 people you meet in Europe or the USA, one of them is likely to have a twin brother or sister. The lowest chance of meeting a twin is in Asia, where 1 in 70 persons is a member of a twin and the highest chance in Nigeria where 1 in 12 persons is a member of a twin pair. In most countries, the twinning rate has steadily increased since the 1980s following a long-term decline in twinning rates from 1900 onwards (Derom *et al*., 1995; Eriksson *et al*., 1995).

There are two types of twins, dizygotic (DZ) and monozygotic (MZ) twins (Hall, 2003), and their etiology is very different. DZ twinning occurs when two separate oocytes are released during the same menstrual cycle (a multiple ovulation event) and fertilized by two sperm. DZ twins, therefore, have the same genetic relationship as ordinary brothers and sisters and on average share 50% of their genes. They can be same sex (boy–boy or girl–girl) or opposite sex (boy–girl) twin pairs. In contrast, MZ twins arise when an embryo splits soon after fertilization. MZ twins carry essentially identical genetic instructions (share 100% of their genes) and are always of the same sex (boy–boy or girl–girl) (Bomsel-Helmreich and Al Mufti, 1995).

The mechanism(s) leading to DZ twins operate on the selection of developing follicles within the ovary where instead of one ovum being released mid-cycle, two follicles mature and both oocytes are released ready for fertilization. For MZ twins, unknown mechanism(s) affect the early development of the embryo immediately after fertilization leading to separation of the cells into two or more embryos. The reasons for the relatively high incidence of MZ twins in humans remain unclear. There are no clear associations between MZ twinning and maternal, environmental or genetic factors and the mechanisms have not been identified, although families with a history of MZ twinning have been reported (Hamamy *et al*., 2004). This review will focus on DZ twinning and recent insights into this intriguing phenomenon. We will discuss the epidemiology of DZ twinning including recent secular trends in twinning rate, the factors affecting DZ twinning in relation to natural selection, the genetics and endocrinology of DZ twinning in humans.

Epidemiology

Prevalence of twinning

Descriptions of variation in the prevalence of twinning for the Nordic Countries were made well before the 19th century. The registration of births began in the 17th century in church registries and was officially introduced in 1749 in Sweden and Finland (Eriksson, 1962). The twinning rate is defined as the number of twin maternities per 1000 maternities and includes still births ($\geq$28 weeks) as well as live births. Thus the total number of

children is twice that of the twin maternities. In the 1970s, Bulmer (1970) divided the geographical distribution of twinning for three large human racial groups from different regions: Europe/North-Africa, Sub-Saharan-Africa and Asia. He described differences in twinning frequencies between these groups, with the highest rate for Sub-Saharan-Africa (~23 per 1000 maternities) and the lowest rate for Asia (~5–6 per 1000 maternities). Little (1988) reviewed the international variation in twinning rate into three groups; low prevalence, intermediate prevalence and high prevalence. The low prevalence group included countries with twinning rates between 2 and 7 per 1000 maternities, such as Hawaii, Japan and Taiwan. Intermediate twinning rates between 9 and 20 per 1000 maternities were seen for most countries in North Africa, America, Asia, Oceania and Europe. The high prevalence group consisted of countries from Africa, especially Nigeria, Seychelles, Transvaal and Zimbabwe, with twinning rates of 20 per 1000 and higher (Nylander, 1979; Little, 1988).

Fluctuations in twinning rates for the USA, Western Europe, Australia and Asia are illustrated in Fig. 1. Data are restricted to the total twinning rate, because twinning rates by sex were not always available. Twinning rates were obtained from literature searches (PubMed, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) and by consulting relevant books and Vital Statistics Reports for twinning rates between 1960 and 2003. Actual data were available for the USA, Australia and The Netherlands. When actual data were not available, we used the twinning rates as reported in the literature. All rates were reported per 1000 maternities allowing the comparison of twinning rates between different countries regardless of the size of their population (Table 1). In most European countries and in the USA, twinning rates started to decline around 1900. However, as shown in Fig. 1 from the 1970s onwards, twinning rates have increased steadily in most countries including the USA, Europe, Australia and Asia (Derom *et al*., 1995; Taffel, 1995; Imaizumi, 1997, 1998). In western European countries, twinning rates rose from 9–11 per 1000 in the 1970s to 15–18 per 1000 by 2001 (Macfarlane *et al*., 2005). Likewise, in Japan, Hong Kong and Singapore the twinning rate rose from 5 to 6 per 1000 births in 1972 to 9 per 1000 births by 2001 (Imaizumi, 2005). Similar increases in twinning rate were also seen for the USA and Australia. The total twinning rate in the USA in 2003 was 15.8 per 1000 maternities. In addition to the total twinning rate, we described the twinning rates for black and white Americans separately because of the heterogeneity of the population in the USA. For black Americans, the twinning rate was 17.2 and for white Americans the twinning rate was 15.7 (compared with twinning rates of 16.1 in Australia and 16.4 in Europe). Twinning rates of Sub-Saharan African countries are not included in Fig. 1 because few data are available. However, Nigeria is often cited in the literature with twinning rates of 40 per 1000 maternities (Little, 1988). Nylander reported twinning rates of 33–66.5 per 1000 maternities in Yoruba women in Western and Eastern Nigeria (Nylander, 1971, 1978) and a twinning rate of 19.4 in Hausa women in the Northern part of Nigeria (Nylander, 1971).

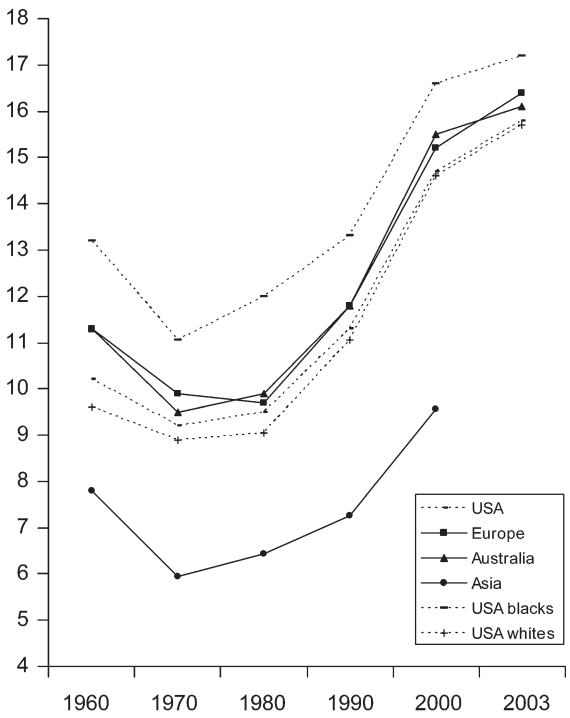

Figure 1: Twinning rates in the USA (total/white/blacks), Europe, Australia and Asia. NB. Twinning rates are presented in terms of twin maternities per 1000 maternities from sources listed in Table 1

Within Europe, twinning rates vary considerably (Fig. 2). In 2003, the average twinning rate in Europe was 16.4 per 1000 maternities, but rates varied widely from country to country from 11 per 1000 maternities in Luxembourg and Portugal to 20 per 1000 in Denmark, Greece and the Netherlands (Hall, 2003; Centraal Bureau, 2004a,b; Macfarlane and Blondel, 2005).

As shown above, twinning rates vary considerably across time and place. As MZ twinning generally occurs at a constant rate of ~4 per 1000 maternities around the world (Tong *et al*., 1997) the variation in twinning rate is generally accepted as being the result of variation in DZ twinning rates.

Contribution of iatrogenic twins in the rising prevalence of twinning

The increased use of fertility treatments such as *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), intra-uterine insemination (IUI) and ovulation induction (OI) is commonly cited as the main cause of the steep increase in twin births in the past two decades (Fauser *et al*., 2005; Martin *et al*., 2005). Lambalk *et al*. (2004) showed however that the increase in opposite sex twins (i.e. DZ twins) in the period 1995–2002 was mainly caused by natural conception (56%) and not by ICSI/IVF (35%) or OI/IUI (9%). Maternal reproductive age was significantly higher in mothers who conceived their twins after natural conception and in mothers who conceived their twins after OI/IUI. In contrast, maternal reproductive age remained the same for mothers who conceived their twins after the use of ICSI or IVF over the period 1995–2002. Lambalk *et al*. (2004) therefore argued that the increase in twin births in the Netherlands was due not only to the use of ART, but also to the increase in maternal reproductive age. It is difficult to say whether maternal age or ART causes the increase in multiple births in the OI/IUI group because maternal age also increased significantly in this group. Tandberg *et al*. (2007) agrees with the general argument that most of the increase in twinning frequency comes from maternal age, but also suggests

Table 1: Summary of studies of twinning rates 1960–2003.

	Period of study	Area studied and remarks
Europe		
Bulmer (1970)	1950–1960	Spain, Portugal, France, Belgium, Austria, Luxembourg, Switzerland, The Netherlands, Germany, Norway, Sweden, Italy, UK (standardized for maternal age)
Derom *et al*. (1995)	1960–2000	Denmark, Netherlands, W-Germany, UK, Luxembourg, Belgium
Eriksson and Fellman (1967, 1973)	1950–1960	Finland, Sweden
Little (1988)	1950–1970	Bulgaria, Ireland
James (1995)	1960–1980	UK, Belgium
Imaizumi (1997)	1972–1996	Austria, Finland, Norway, Sweden
Astolfi *et al*. (2003)	1975–1995	Italy, Norway, Sweden, Austria, UK
Centraal Bureau voor de Statistiek (2004a,b)	1950–2003	The Netherlands
Macfarlane and Blondel (2005)	1998–2002	Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, The Netherlands, Portugal, Spain, Sweden, UK
USA		
Bulmer (1970)	1950–1960	USA (American whites and blacks)
Terry (1962)	1960	USA, birth records of NCHS
Little (1988)	1978–1979	USA, not specified
Jewell and Yip (1995)	1980–1989	USA, birth records of NCHS
Martin and Park (1999)	1980–1997	National Vital Statistics Report (NCHS)
Kiely and Kiely (2001)	1971–1998	USA, birth records of NCHS
Martin *et al*. (2002)	2000	National Vital Statistics Report (NCHS)
Mathews and Hamilton (2002)	2002	Mean age of mother, 1970–2000 (NCHS)
Martin *et al*. (2005)	2003	National Vital Statistics Report (NCHS)
Markovitz and Hershlag (2005)	1997–2001	SART-CDC Registry
Australia		
Australian Bureau of Statistics (2001)	1980–2000	Births, Australia, 2000, No. 3301.0
Umstad and Lancaster (2005)	1983–1999	Australia
Australian Bureau of Statistics (2005)	1985–2005	Births No. 3301.0
Asia		
Bulmer (1970)	1960–1970	Japan, China
Little (1988)	1960–1980	Japan, Hong Kong, Singapore
Imaizumi (2005)	1970–2001	Japan, Hong Kong, Singapore
Africa		
Bulmer (1970)	1960	Bantu, West-Africa
	1960	South-Africa, Ghana
Nylander (1969, 1979)	1970	Nigeria

age and ART does not account for all the increase. Recently Jones (2007) showed that in the USA in 2003 the greatest number of twin births resulted from natural conception (60.1%), but a very large number of twins are also born after OI/IUI (31.6%). However, he did not control for maternal age which means that the percentages of spontaneous twin births could be underestimated and the percentages of OI/IUI births overestimated.

Factors affecting twinning

Major factors influencing twinning are maternal age, parity and genetic inheritance. An important early contribution to the study of twinning in relation to maternal characteristics was made in 1865 by the Scottish physician Matthews Duncan, who reported an increase in twin pregnancies with an increase in maternal age (Bulmer, 1970). Duncan also described an increased risk of twinning with increased parity, the number of children born to the mother prior to the twin pregnancy. Although maternal age and parity are highly correlated, the effects are independent of each other (Bulmer, 1970). It was later discovered that these factors predominantly influence the DZ twinning rate, and not the MZ twinning rate (Bulmer, 1970; MacGillivray *et al*., 1988). DZ twinning rate increases 4-fold from age 15 to 35 (Bulmer, 1970). The decline in the DZ twinning rate in the early 20th century reflected a decrease in mean maternal age and a lower number of maternities. The increase in twinning rate reported in the late 1970s has been mainly associated with an older age at childbearing (Derom *et al*., 1995; Lambalk *et al*., 2004). The increased use of fertility treatments such as *in vitro* fertilization (IVF) in the late 1980s further added to the rising incidence in twin births (Fauser *et al*., 2005; Martin *et al*., 2005).

Body composition has been linked to twinning. Nylander showed that for tall women (164 cm and over), the relative risk of having twins was 1.5–2.0 times higher than for short women (under 155 cm), after standardization for age and parity (Nylander, 1981). Other research found a direct, but somewhat inconsistent association between increased maternal height and DZ twinning (Bortolus *et al*., 1999). Recently, Basso and colleagues found a similar trend (Basso *et al*., 2004). Twin mothers were taller and had a higher body mass index (BMI) compared to mothers of singleton children. A BMI of less than 20 was associated with a lower risk of twinning and a BMI of 30 or more was associated with a higher risk of twinning. These results remained after correcting for maternal age and parity (Basso *et al*., 2004).

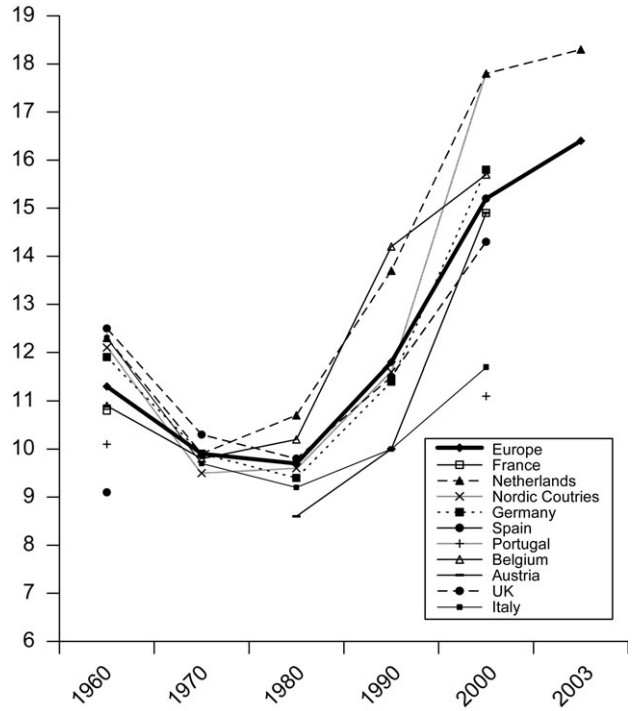

Figure 2: Twinning rates in Europe. Twinning rates are presented in terms of twin maternities per 1000 maternities from sources listed in Table 1

Significantly higher multiple birth rates have been reported in mothers who smoke (Olsen *et al*., 1988; Parazzini *et al*., 1996), although insufficient control for covariates (Olsen *et al*., 1988) and limited power (Parazzini *et al*., 1996) suggest caution in interpretation of these findings (Kallen, 1998). A recent prospective study in Denmark supports a small effect of smoking on twinning (Morales-Suarez-Varella *et al*., 2007).

Associations with socio-economic status (SES) have also been reported (MacGillivray and Campbell, 1978; MacGillivray *et al*., 1988) but findings on differences in twinning by SES are often difficult to interpret. This may be due to ambiguity of definition and because of its interrelation with factors associated with SES, like height, weight, smoking behavior and age of the mother at birth of the children (Campbell, 2005).

Seasonal variation influences DZ twinning. Higher rates of DZ twinning are reported for conceptions during summer and autumn in several countries (Dionne *et al*., 1993; Sharma, 1997; Fellman and Eriksson, 1999; Eriksson and Fellman, 2000), although this trend is not seen in all studies (Bonnelykke *et al*., 1987; Krieger *et al*., 1996). The seasonal variation appears stronger for data from the 19th century compared with recent trends (Fellman and Eriksson, 1999; Eriksson and Fellman, 2000). Seasonal variation in day length may influence hormonal concentrations driving ovarian activity and influence fertility and multiple ovulation (Dionne *et al*., 1993). Seasonal changes in food supply may also have contributed to stronger effects in the past (Eriksson and Fellman, 2000).

Two iatrogenic factors; the use of oral contraceptives and folic acid are also reported to influence DZ twinning (Ericson *et al*., 2001; Lumley *et al*., 2001; Li *et al*., 2003). Some studies found an increase in twin births associated with the consumption of folic acid around the time of conception (Ericson *et al*., 2001; Kallen, 2004; Vollset *et al*., 2005), whereas others were not able to replicate these findings (Li *et al*., 2003; Berry and Kihlberg, 2005). The issue is important since large scale preconception use of folic acid has been introduced for the prevention of neural tube defects (Botto *et al*., 1996) and folic acid may increase the chance of successful implantation and survival of two embryos (Lumley *et al*., 2001). No clear relationship has been established and data may be confounded by fertility treatment and maternal age (Levy and Blickstein, 2006). Recently a systematic review evaluated 12 studies to examine if the preconception use of folic acid increases the risk of twinning (Muggli and Halliday, 2007). They concluded there is some evidence for a positive relationship between preconception folic acid use and twinning. However, the relationship is tentative and additional well-designed studies are required that focus on the dose relationship and obtain accurate data on infertility treatments (Muggli and Halliday, 2007).

Earlier studies investigating a possible association between oral contraceptives and twinning found a decrease in DZ twinning rates after the use of oral contraception and sometimes an increase in MZ twinning rates (Macourt *et al*., 1982). In 1977, Rothman evaluated the effect of oral contraceptives on reproduction (Rothman, 1977). Twinning was more frequent when mothers became pregnant soon after they stopped taking oral contraceptives, and the increase was mainly caused by DZ twins. A large study in Aberdeen found no association between the use of oral contraception and either MZ or DZ twinning (Campbell *et al*., 1987). In this study, zygosity was determined from blood samples and placentation, and data regarding oral contraceptive use was collected prior to the twin pregnancy with three control groups. A more recent study however did find an increased risk of twinning after discontinuation of the oral contraceptive pill (Murphy *et al*., 1989).

A theoretical reason for an increased risk of DZ twinning after taking oral contraceptives is that the hypothalamic-pituitary-ovarian axis (HPO-axis) has to recover from the effects of exogenous steroids, causing a temporary increase of FSH levels (Jernstrom *et al*., 1995). A clinical situation that mimics such a condition is when women with hypothalamic amenorrhea are treated with GnRH to induce ovulation where in the first treatment only this leads to higher FSH levels in association with multiple follicle growth and an increased risk of multiple pregnancies (Lambalk *et al*., 1998b).

Genetic factors

The initial link between body composition and twinning was made by Tchouriloff in 1877. He argued that taller women are more likely to bring a twin pregnancy to full-term because of their body size, and are therefore predisposed to conceive twins (MacGillivray *et al*., 1988). As height was already known to be an inherited characteristic, this was the first attempt to link twinning with heredity. Later Weinberg (1901) discovered familial clustering of DZ twin pregnancies. He found that mothers, sisters and daughters of mothers with multiples, had increased risks of conceiving a multiple by 39, 95 and 30%, respectively. However, he could not find this increased risk in relatives on the paternal side. Furthermore, he compared the twinning rate among the relatives of mothers of opposite and same sex twins, using his differential method (Weinberg, 1934). This method is based on the numbers of same and opposite sex twins. According to this method, the DZ twinning rate (DZr) can be estimated by doubling

the number of opposite sex twins (OS) and dividing the number by the total number of maternities (*N*) (DZr = 2OS/N). The MZ twinning rate (MZr) can be estimated by subtracting the number of OS twins from the number of same sex (SS) twins and dividing the number by the total number of maternities (*N*) (MZr = (SS − OS)/*N*). He found twinning rates of 22.0 and 11.1, respectively, in contrast to a twinning rate of 11.9 in the general population. Weinberg therefore suggested that the inheritance of DZ twinning was restricted to the female line. In 1934, Greunlich disputed this as he found that the paternal side was as important as the maternal side in determining the twinning rate in siblings of fathers and mothers of twins (Greunlich, 1934). Later, Wyshak and White, however, analysed the inheritance of twinning using data obtained from the archives of the Genealogical Society of the Mormon Church in Salt Lake City and concluded that DZ twinning is determined by recessive genes, and limited to the female side (White and Wyshak, 1964; Wyshak and White, 1965). Further support was obtained from a study by Bulmer (1970) showing that only relatives of mothers of twins report significantly higher twinning rates in other family members. In this study, the risk of conceiving a twin was 1.7 times higher in female relatives than in male relatives. Looking in more detail at the DZ twinning rate among female relatives, he showed that the risk of having a DZ twin is 2.5 times higher than the twinning rate in the general population for women with a sister with DZ twins. For mothers and daughters of a mother with a DZ twin this was about twice as high as the risk for the general population (Bulmer, 1970). Studies in Italy reported significantly increased twinning in maternal relatives and some evidence for an increase among paternal relatives of DZ twins (Parisi *et al*., 1983).

More recently, Meulemans and colleagues (1996) investigated the inheritance of DZ twinning in 1422 Dutch and Flemish pedigrees of mothers of spontaneous DZ twins by formal pedigree analysis. Analysis showed that the phenotype of "having DZ twins" is consistent with an autosomal monogenic dominant model. This finding means that the phenotype of giving birth to a twin is expressed in women, but can be inherited from both the maternal and the paternal side. In the same period, Lewis and colleagues (1996) investigated frequencies of other twins in families of 6596 twin pairs from the Australian Twin Registry. They found a relative risk of 1.7 and 2.5, respectively, for sisters of mothers of DZ twins and offspring of female DZ twins. The evidence strongly suggests that genetic contribution to DZ twinning is a trait expressed by the mother that may be inherited from either parent. Limited evidence for a paternal effect was recently supported by a small study showing semen quality of the father may play some role in twinning (Asklund *et al*., 2007). DZ twinning is influenced by many genes (see below) and unlikely to be a simple dominant or recessive trait.

Natural selection for twinning

Twinning is often associated with complications during pregnancy and delivery for both the mother and the twins (Gabler and Voland, 1994; Conde-Agudelo *et al*., 2000; Elster, 2000). So why do women have twins, when the costs are so high? Two hypotheses have been put forward to explain the human twinning rate; the 'the insurance ova hypothesis' (Anderson, 1990) and the 'natural selection hypothesis' (Lummaa *et al*., 1998). The insurance ova hypothesis states that producing more then one ovum increases the probability that at least one will be fertilized and survive to term. Conceiving twins is therefore a by-product of selection for increased fertility (Anderson, 1990). The second hypothesis views twinning as either adaptive, or maladaptive, depending on the environment (Lummaa *et al*., 1998). Lummaa and colleagues investigated their natural selection hypothesis by comparing the lifetime reproductive success between singleton and twin mothers living on the archipelago of Åland and Åboland and the mainland of Finland. In the food rich archipelago, lifetime reproductive success was maximized for mothers having twins. In contrast, in the poorer mainland environment, lifetime reproductive success was maximized by having singletons (Lummaa *et al*., 1998). The fall in DZ twinning rate associated with malnourishment in World War II and subsequent increase in DZ twinning rates when food availability became stable again (Bulmer, 1970) provide further support for this 'natural selection' hypothesis.

Helle and colleagues contrasted both hypotheses in three historical Sami populations from Northern Scandinavia. They found that twin mothers had a higher overall reproductive success compared with singleton mothers. Mothers of twins started reproduction at a younger age, and continued for longer; they had higher lifetime fecundity and raised more offspring to adulthood in all studied populations. However, if the mother begun to reproduce at a very late age (>37 years), or if she had a long reproductive life span (>20 years), it was more favorable to produce singletons (Helle *et al*., 2004). This finding indicates that women predisposed to having twins are more likely to produce twins in a healthy reproductive environment (Helle *et al*., 2004).

Mechanisms controlling DZ twinning

It is generally accepted that hereditary DZ twinning results from the fertilization of two separate oocytes by two sperm (Hall, 2003). The increase in multiple ovulation (and twinning frequency) resulting from improved nutrition or specific genetic mutations is well documented for other species (Montgomery *et al*., 1988, 1993; Hunter *et al*., 2004). Direct observations of increased multiple ovulation in human twinning have not been made. However, increased follicle recruitment has been observed in mothers of spontaneous twins (Martin *et al*., 1991b) and similar genome-wide allele sharing for DZ twins and sibs (Montgomery *et al*., 2006) supports the view that DZ twins arise from multiple ovulation and subsequent fertilization and survival.

Growth and selection of follicles destined for ovulation is controlled by a complex regulatory network within the hypothalamic-pituitary-ovarian axis (Fig. 3). Two hormones from the pituitary gland are essential for reproductive function. These are the two gonadotrophins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and the concentrations of both FSH and LH change in characteristic patterns during each menstrual cycle. FSH concentrations begin to increase at the beginning of each cycle starting a new wave of follicle growth among the larger follicles in the growing pool. In turn, the growing follicles secrete hormones that signal back to the brain and pituitary gland causing FSH concentrations to fall. Few follicles can sustain

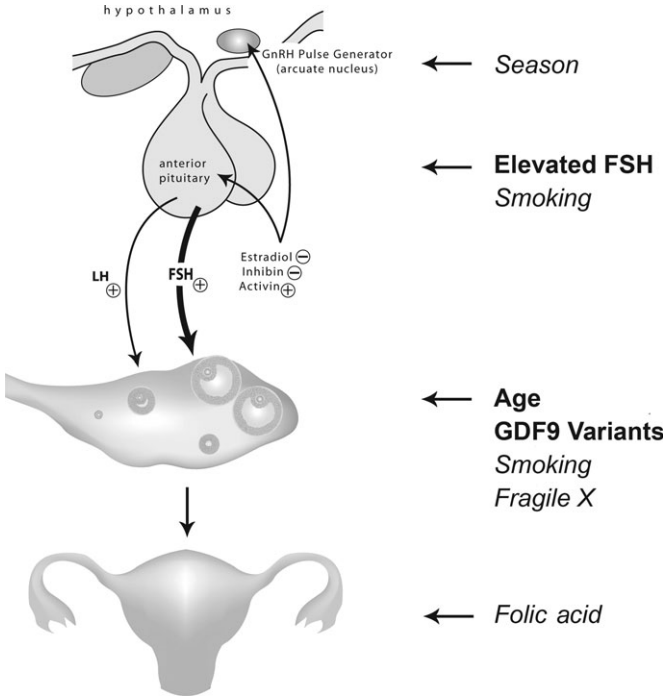

Figure 3: Regulatory factors in the growth and selection of follicles GDP9 = growth differentiating factor 9

growth in a time of falling FSH concentrations (McGee and Hsueh, 2000). Usually, development of one dominant follicle takes place when FSH concentrations reach a required threshold early in each cycle (Brown, 1978; Baird, 1987). Multiple follicle growth results when greater FSH concentrations occur at the time of follicle selection or when FSH concentrations exceed the threshold for too long (Baird, 1987; Schoemaker *et al*., 1993).

Recent studies have defined a complex signaling network within the ovarian follicle itself (Shimasaki *et al*., 2004; Roy and Matzuk, 2006). This pathway responds to the external FSH and LH signals and ensures coordinated growth and development of the oocyte and other follicle cell types. Major players include two closely related growth factors expressed specifically in the oocyte, namely bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) (Shimasaki *et al*., 2004). These growth factors bind to the specific receptors expressed on multiple cells in the ovary ensuring that the oocyte is supported during growth and development.

Mothers of DZ twins have elevated FSH concentrations (Nylander, 1974; Martin *et al*., 1984; Lambalk *et al*., 1998a,b) suggesting that factors influencing twinning may operate through an increase in secretory drive from the hypothalamic-pituitary system. However, increased FSH concentrations have not been observed in all studies (Gilfillan *et al*., 1996). There is evidence for an increase in the number of FSH pulses in the early follicular phase in mothers of DZ twins (Lambalk *et al*., 1998a). These FSH pulses occur without a concurrent LH pulse. LH secretion and both the LH and the FSH responses from the pituitary to gonadotrophin releasing hormone (GnRH) were not different (Lambalk *et al*., 1998a).

FSH release is regulated by feedback from inhibin peptides in the ovary (Fig. 3). Inhibin produced by the ovary acts as a classic hormone at the level of the pituitary by inhibiting pituitary FSH synthesis and release (Baird and Smith, 1993). There are two forms of inhibin, inhibin A and inhibin B; both forms consist of two sub-units, a common α-subunit (INHA) and either β_a or β_b (INHBA and INHBB, respectively) (Martin *et al*., 1991a; Burger *et al*., 1995). No differences in concentrations of inhibin A or B were detected across the menstrual cycle between mothers of DZ twins and controls (Gilfillan *et al*., 1996; Lambalk *et al*., 1998a) suggesting no difference in the mode of ovarian feedback on FSH concentrations. The data indicate that neuroendocrine mechanisms may be involved in the generation of higher FSH levels in hereditary twinning (Martin *et al*., 1984; Lambalk *et al*., 1998a).

The age related increase in the frequency of DZ twins

The chance of having DZ twins increases approximately 4-fold with increasing maternal age (Bulmer, 1970). The reason for the increase in twinning frequency with age is thought to lie in the dynamic interplay of hormonal signals between the pituitary gland and the ovary (Lambalk *et al*., 1998b). In younger women, there is usually a pool of growing follicles ready to respond immediately to the rise in FSH at the beginning of each menstrual cycle. The immediate response is to send hormonal signals back to the brain and pituitary gland to turn down the FSH signal (Baird, 1983; Baker and Spears, 1999; Macklon and Fauser, 2000; Zeleznik, 2001). This stops the growth of other follicles and the one dominant follicle usually goes on to ovulate at mid-cycle.

With age, the pool of ovarian follicles available to grow and respond to the hormonal signals diminishes (Lambalk *et al*., 1998b). Consequently, when FSH rises at the beginning of each cycle, large follicles may not be available to respond rapidly to this signal. Sometimes hormonal feedback from two smaller follicles needs to be combined before the message gets through to turn down the FSH signal. When this happens both follicles mature and ovulate increasing the chance of having twins. As the pool of growing follicles decreases further, the feedback signal diminishes and the background concentration of FSH rises, further increasing the chance of two follicles ovulating and giving rise to twins (Lambalk *et al*., 1998b). Thus the increase in DZ twinning with age is thought to be due to rising FSH concentrations driving the selection of more ovarian follicles.

Genetic variants contributing to DZ twinning

One approach to better understand mechanisms contributing to variation in twinning is to identify the gene or genes that account for the genetic variation in DZ twinning. Given our current understanding of mechanisms contributing to ovarian follicle selection and twinning, an obvious place to look is within the complex regulatory network of the hypothalamic-pituitary-ovarian axis (Fig. 3). Candidate genes include the genes coding for FSH and the specific transmembrane receptor for FSH (FSHR). Al-Hendy and colleagues suggested that two linked mutations (Thr307Ala and Asn680Ser) causing a higher sensitivity of the FSH receptor to FSH, may contribute to variation in twinning (Al Hendy *et al*., 2000). However, other researchers could not agree with the conclusions of these authors, stating that it is very doubtful the mutations are responsible for variation in DZ

twinning (Derom *et al*., 2001; Gromoll and Simoni, 2001; Liao *et al*., 2001). Montgomery and colleagues sequenced the transmembrane region of the FSHR in 21 unrelated mothers of DZ twins and found no association. Furthermore, a linkage study of 183 sister pairs with spontaneous DZ twins excluded linkage to the region on chromosome 2 where FSHR is located which suggest that mutations in FSHR are not a common cause of hereditary twinning (Montgomery *et al*., 2001a).

Polymorphisms in the INHA gene located on chromosome 2 in humans were typed in DZ twinning families (Montgomery *et al*., 2000). There was no evidence for association between variation at the α-inhibin locus and DZ twinning. Although the inhibin B form of inhibin could be an interesting candidate to investigate for association with DZ twinning, the authors also suggest that mutations in other candidates on chromosome 2, like the β_b-inhibin subunit cannot be major contributors to risk for DZ twinning (Montgomery *et al*., 2000).

Lessons from animal studies

The number of offspring is an important economic trait in farmed animals and factors influencing variation in twinning and litter size have been studied extensively. Sheep provide a valuable model because most breeds have singles or twins, but some strains have a high incidence of triplets and higher order multiples (Montgomery *et al*., 1992, 2001b). This high frequency of twins and triplets is influenced by genetic background. Studies of one of these strains 30 years ago showed that the high litter size resulted from the actions of a single gene (Montgomery *et al*., 1992). At the time this was a surprise because genetic effects on twinning were thought to result from the actions of many genes with small effects rather than the actions of one or two genes (or variants) of large effect. The discovery sparked a worldwide search to find the gene responsible and to look for other sheep strains with single genes affecting twinning (Montgomery *et al*., 2001b; Moore *et al*., 2004).

Both searches were successful. Mutations have been found in three different genes that increase the frequency of twinning (Galloway *et al*., 2000; Mulsant *et al*., 2001; Souza *et al*., 2001; Wilson *et al*., 2001; Hanrahan *et al*., 2004). Two of these genes are closely related growth factors expressed specifically in the oocyte and known as bone morphogenetic protein 15 (BMP15) (Galloway *et al*., 2000) and growth differentiation factor 9 (GDF9) (Hanrahan *et al*., 2004). The third gene is the receptor for BMP15 expressed on multiple cells in the ovary and known as bone morphogenetic protein receptor 1B (BMPR1B) (Wilson *et al*., 2001; Mulsant *et al*., 2001). The mutations increasing twinning in sheep are all found in these three genes within the ovary suggesting, at least for this species, the primary control of the number of ovulated follicles and hence twinning frequency resides within the ovary itself (Moore *et al*., 2004).

Some of these mutations are also associated with infertility (Galloway *et al*., 2000; Hanrahan *et al*., 2004), when individuals carry two copies of the mutations. Infertility in sheep for some mutations in BMP15 and GDF9 is recessive. Carriers of one copy of the mutation have more twins, whereas carriers with two non-functional copies of the gene exhibit a failure of normal ovarian development and are infertile.

The role of genes from ovarian regulatory pathways in human DZ twinning

GDF9 and BMP15 are both expressed in human oocytes and play important roles in folliculogenesis. The role of common variation in GDF9 influencing DZ twinning was tested by genotyping markers across the gene in families with spontaneous DZ twins (Montgomery *et al*., 2004). Six common alleles of human GDF9 were identified with no evidence for association with DZ twinning. DNA sequencing in 20 mothers of DZ twins found a loss of function mutation on one copy of the GDF9 gene in one family (Montgomery *et al*., 2004). The mutation was present in two sisters with spontaneous DZ twins and inherited from their father. A further search for GDF9 variants in mothers of spontaneous DZ twins was carried out in additional families (Palmer *et al*., 2006). Two novel deletions and four missense alterations in GDF9 were identified in mothers of DZ twins. One deletion mutation was identified in two families and in each family was carried by two sisters with DZ twins including one individual with three sets of DZ twins. Taken together, the frequency GDF9 variants were significantly higher in mothers of DZ twins compared to controls. Variants contributing to increased twinning are summarized in Fig. 4 and appear to double the chance of having twins for women who carry the variants. However, the frequency of the variants is low (<4% for all variants) and so the contribution of these variants to the overall incidence of twinning is small.

The search for other genes

Several groups are collecting families with a high incidence of twinning to identify genes contributing to twinning variation. The standard method to search for genes is to follow the inheritance of fragments across all chromosomes in the families and look for chromosome segments more often inherited together in mothers of twins. One candidate for multiple ovulation and increased fertility is the protease inhibitor locus (Pi). Analysis of Pi types in twins suggested that intermediate antitrypsin deficiency was more common in twins and parents of twins and increased fertility may provide a selective advantage to maintain polymorphism at this locus (Lieberman *et al*., 1978). In a sample of 160 Dutch twin families, mothers of DZ twins carried the S and the Z allele at the protease inhibitor (Pi) three times more frequently than a control sample (Boomsma *et al*., 1992). It was argued that the S allele might increase the risk of multiple ovulations and increase multiple gestations, because mothers of MZ twins also had an increased frequency on the S allele.

Almost 20 years ago, female carriers of the fragile X (FRAXA) syndrome were found to have an increased risk of DZ twin pregnancies (Fryns, 1986; Kenneson and Warren, 2001). Three classes of the gene (FMR1) are defined by the number of CGG repeats.

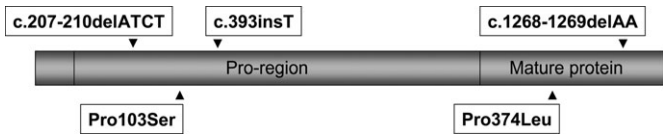

Figure 4: Insertion/deletion and missense mutations reported in growth differentiation factor (GDF9) that are associated with an increase in twinning frequency (Montgomery *et al*., 2004; Palmer *et al*., 2006)

Individuals with less than 60 CGG repeats have a normal gene. Individuals with 55–200 CGG repeats have a premutation which means they carry an unstable mutation which can expand in future generations. Individuals with over 200 repeats have a full mutation which causes fragile X syndrome. Carriers of the FMR1 premutation are at risk for POF, early menopause, ovarian dysfunction and an increase in DZ twinning (Schwartz *et al*., 1994; Turner *et al*., 1994; Vianna-Morgante, 1999; Welt *et al*., 2004). However, Healey *et al*. (1997) found no premutations or full mutations in mothers of spontaneous DZ twins and concluded that FMR1 can play no more than a minor role in the inheritance of DZ twinning. Associations with twinning may relate to earlier ovarian aging in premutation carriers (Welt *et al*., 2004).

Recently, Busjahn and colleagues (2000) reported that a C→T substitution allele in the gene PPRAG on chromosome 3p25 encoding peroxisome proliferator-activated receptor (PPARγ) is associated with DZ twinning. This gene might also be involved in the intra-uterine selection and thus survival of the unborn twins (Busjahn *et al*., 2000). Duffy and colleagues (2001a) could not replicate linkage at the PPRAG gene region for either survival of twin pregnancy or multiple ovulation.

Recently a genome-wide linkage scan for natural DZ twinning was conducted in 14 Flemish families. The observed peaks were highest under a dominant model of inheritance, with LOD scores of 1.51, 1.36 and 1.99 found on chromosome 2, 7 and 18, respectively (Derom *et al*., 2006). These chromosomes may contain genes contributing to the variation in DZ twinning, but clarification awaits further study. The results also allowed examination of evidence for contributions to DZ twinning from common variation at many of the candidates discussed above, including genes from the pituitary-ovarian axis implicated in control of DZ twinning (Fig. 3). A role for many of these genes was excluded (Derom *et al*., 2006). These conclusions agree with previous studies for inhibin alpha (INHA; Montgomery *et al*., 2000), FSH receptor (FSHR) (Montgomery *et al*., 2001a), bone morphogenetic protein receptor 1B (BMPR1B) (Duffy *et al*., 2001b) and methylenetetrahydrofolate reductase (MTHFR) (Montgomery *et al*., 2003). There was no evidence for linkage in the region of GDF9 (Derom *et al*., 2006), despite the recent evidence for mutations in GDF9 increasing the chance of having twins (Palmer *et al*., 2006). However, the frequency of these rare GDF9 mutations is low. They contribute only a small proportion to the variation in twinning and the signal would not be picked up in the linkage scan. It is therefore possible that rare mutations in other candidates could still contribute to twinning in some families.

Relationship between twinning and premature ovarian failure

GDF9 and BMP15 are critical genes for normal human fertility (Di Pasquale *et al*., 2004, 2006; Dixit *et al*., 2005, 2006). A dominant-negative mutation in BMP15 identified in Italian sisters causes ovarian dysgenesis (Di Pasquale *et al*., 2004) and recent studies found higher frequencies of rare mutations in both GDF9 and BMP15 in patients with premature ovarian failure (POF) when compared with controls (Dixit *et al*., 2005, 2006; Di Pasquale *et al*., 2006; Laissue *et al*., 2006; Kovanci *et al*., 2007). POF is diagnosed when women younger than 40 years have unexplained amenorrhea for longer than 6 months, have high FSH levels and low estrogen levels (Coulam *et al*., 1986).

Mutations in GDF9 in mothers of DZ twins and in patients with POF imply a possible direct relationship between twinning and POF. Mothers of DZ twins reach menopause significantly earlier than mothers of MZ twins (Martin and Park, 1999; Gosden *et al*., 2007). The small increase in the frequency POF in mothers of DZ twins could be explained by mutations in GDF9 and BMP15 influencing both aspects of ovarian function. The variants detected in GDF9 in mothers of twins (Palmer *et al*., 2006) were different from variants in POF patients in India (Dixit *et al*., 2005), although this may reflect the different populations used for the studies. Future studies should examine whether the same variants in GDF9 and BMP15 affect both twinning and POF via common mechanisms or whether different variants affect these two aspects of human fertility.

Summary

MZ and DZ twins arise through different mechanisms. Mechanisms for MZ twinning remain obscure, but both types of twinning probably have their origins in the complex regulation of growth and development of the ovarian follicle and early embryo. More is known about the factors involved in DZ twinning. Maternal age has played a major role in fluctuations in twinning frequency during the last 100 years following changing demographic trends. Other factors including body composition, height, seasonality and smoking also seem to contribute to the variation in DZ twinning frequency.

Genetic background contributes significantly to variation in DZ twinning both between and within populations. The known effects of maternal age and genetic variation on DZ twinning suggest that in women the number of follicles that ovulate is controlled by both external hormonal drive and growth factor signaling within developing follicles. Genetic mapping studies in humans and other species are beginning to identify the genes and pathways contributing to the variation in DZ twinning. Associations with α-1-antitrypsin and FMR1 should be regarded as tentative and effects of FMR1 may be indirect through accelerated ovarian aging. Recent studies have shown that mutations in the oocyte specific growth factor GDF9 contribute to both DZ twinning and to premature ovarian failure. Future studies will examine whether variants in GDF9 and BMP15 affect both twinning and POF via common mechanisms or whether different variants affect these two aspects of human fertility. Greater understanding of the causes of DZ twinning may provide new insights into mechanisms influencing both fertility and infertility.

Funding

Supported by the National Institute of Child Health and Human Development (Grant nr HD042157).

References

Al Hendy A, Moshynska O, Saxena A, Feyles V. Association between mutations of the follicle-stimulating-hormone receptor and repeated twinning. *Lancet* 2000;**356**:914.

Anderson DJ. On the Evolution of Human Brood Size. *Evolution* 1990;**44**: 438–440.

Asklund C, Jensen TK, Jorgensen N, Tabor A, Sperling L, Skakkebaek NE. Twin pregnancy possibly associated with high semen quality. *Hum Reprod* 2007;**22**:751–755.

Astolfi P, Ulizzi L, Zonta LA. Changes in twinning rate: Italy 1950–1996. *Hum Reprod* 2003;**18**:207–211.

Australian Bureau of Statistics. 2001; Births, Australia, 2000 (Rep. No. Cat. No. 3301.0).

Australian Bureau of Statistics. 2005; Births (Rep. No. 3301.0).

Baird DT. Factors regulating the growth of the preovulatory follicle in the sheep and the Human. *J Reprod Fert* 1983;**69**:343–352.

Baird DT. A model for follicular selection and ovulation: lessons from superovulation. *J Steroid Biochem* 1987;**27**:15–23.

Baird DT, Smith KB. Inhibin and related peptides in the regulation of reproduction. *Oxf Rev Reprod Biol* 1993;**15**:191–232.

Baker SJ, Spears N. The role of intra-ovarian interactions in the regulation of follicle dominance. *Hum Reprod Update* 1999;**5**:153–165.

Basso O, Nohr EA, Christensen K, Olsen J. Risk of twinning as a function of maternal height and body mass index. *JAMA* 2004;**291**:1564–1566.

Berry RJ, Kihlberg R. Folic acid supplementation is associated with an increase in dizygotic twinning. *Early Hum Dev* 2005;**81**:465–467.

Bomsel-Helmreich O, Al Mufti W. The mechanism of monozygosity and double ovulation. In: Keith LG, Papiernik E, Keith DM, Luke B (eds). *Multiple pregnancy*, 1st edn., London, UK: The Parthenon Publishing GroupLondon, UK, 1995, 25–40.

Bonnelykke B, Sogaard J, Nielsen J. Seasonality in twin birth rates, Denmark, 1936–84. *J Epidemiol Community Health* 1987;**41**:338–343.

Boomsma DI, Frants RR, Bank RA, Martin NG. Protease inhibitor (Pi) locus, fertility and twinning. *Hum Genet* 1992;**89**:329–332.

Bortolus R, Parazzini F, Chatenoud L, Benzi G, Bianchi MM, Marini A. The epidemiology of multiple births. *Hum Reprod Update* 1999;**5**:179–187.

Botto LD, Khoury MJ, Mulinare J, Erickson JD. Periconceptional multivitamin use and the occurrence of conotruncal heart defects: results from a population-based, case-control study. *Pediatrics* 1996;**98**:911–917.

Brown JB. Pituitary control of ovarian function concepts derived from gonadotrophin therapy. *Aust NZ J Obstet Gynaecol* 1978;**18**:46–54.

Bulmer MG. *The Biology of Twinning in Man*. Oxford, UK: Oxford Clarendon PressOxford, UK, 1970.

Burger HG, Farnworth PG, Findlay JK, Gurusinghe CJ, Healy DL, Mamers P, Mason A, Robertson DM. Aspects of current and future inhibin research. *Reprod Fertil Dev* 1995;**7**:997–1002.

Busjahn A, Knoblauch H, Faulhaber HD, Aydin A, Uhlmann R, Tuomilehto J, Kaprio J, Jedrusik P, Januszewicz A, Strelau J *et al*. A region on chromosome 3 is linked to dizygotic twinning. *Nat Genet* 2000;**26**: 398–399.

Campbell D, Thompson B, Pritchard C, Samphier M. Does the use of oral contraception depress DZ twinning rates. *Acta Genet Med Gemellolog* 1987;**36**:409–415.

Campbell DM. Natural Factors Influencing Multiple Gestation: Perspectives from long-term observations in Scotland. In: Blickstein I, Keith LG, Keith DM (eds). *Multiple Pregnancy* 2nd edn. London and New York: Taylor and Francis GroupLondon and New York, 2005, 87–93.

Centraal Bureau voor de Statistiek. Aantal tweelinggeboorten in 25 jaar verdubbeld, 2004a. http://www.cbs.nl/nl-NL/menu/themas/bevolking/publicaties/artikelen/archief/2004/2004-1513-wm.htm://www.cbs.nl/nl-NL/menu/themas/bevolking/publicaties/artikelen/archief/2004/2004-1513-wm.htm.

Centraal Bureau voor de Statistiek. Geboorte naar diverse kenmerken, 2004b. http://statline.cbs.nl/StatWeb/table.asp?HDR=T&LA=nl&DM=SLNL&PA=37422ned&D1=0,4-5,7,9,11,13,17,26,35,40-41&D2=0,10,20,30,40,(l-4)-l&STB=G1.

Conde-Agudelo A, Belizan JM, Lindmark G. Maternal morbidity and mortality associated with multiple gestations. *Obstet Gynecol* 2000;**95**:899–904.

Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. *Obstet Gynecol* 1986;**67**:604–606.

Derom C, Groenen P, Vlietinck R. Follicle-stimulating-hormone receptor and twinning. *Lancet* 2001;**357**:230–231.

Derom C, Jawaheer D, Chen WV, McBride KL, Xiao X, Amos C, Gregersen PK, Vlietinck R. Genome-wide linkage scan for spontaneous DZ twinning. *Eur J Hum Genet* 2006;**14**:117–122.

Derom R, Orlebeke J, Eriksson A, Thiery M. The epidemiology of multiple births in Europe. In: Keith LG, Papiernik E, Keith DM, Luke B (eds). *Multiple pregnancy*, 1st edn. London: The Parthenon Publishing GroupLondon, 1995,145–162.

Di Pasquale E, Beck-Peccoz P, Persani L. Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (BMP15) gene. *Am J Hum Genet* 2004;**75**:106–111.

Di Pasquale E, Rossetti R, Marozzi A, Bodega B, Borgato S, Cavallo L, Einaudi S, Radetti G, Russo G, Sacco M *et al*. Identification of new variants of human BMP15 gene in a large cohort of women with premature ovarian failure. *J Clin Endocrinol Metab* 2006;**91**:1976–1979.

Dionne CE, Soderstrom M, Schwartz SM. Seasonal variation of twin births in Washington State. *Acta Genet Med Gemellol (Roma)* 1993;**42**:141–149.

Dixit H, Rao LK, Padmalatha V, Kanakavalli M, Deenadayal M, Gupta N, Chakravarty B, Singh L. Mutational screening of the coding region of growth differentiation factor 9 gene in Indian women with ovarian failure. *Menopause* 2005;**12**:749–754.

Dixit H, Rao LK, Padmalatha V, Kanakavalli M, Deenadayal M, Gupta N, Chakrabarty B, Singh L. Missense mutations in the BMP15 gene are associated with ovarian failure. *Hum Genet* 2006;**119**:408–415.

Duffy D, Montgomery G, Treloar S, Birley A, Kirk K, Boomsma D, Beem L, de Geus E, Slagboom E, Knighton J *et al*. IBD sharing around the PPARG locus is not increased in dizygotic twins or their mothers. *Nat Genet* 2001a;**28**:315.

Duffy DL, Montgomery GW, Hall J, Mayne C, Healey SC, Brown J, Boomsma DI, Martin NG. Human twinning is not linked to the region of chromosome 4 syntenic with the sheep twinning gene FecB. *Am J Med Genet* 2001b;**100**:182–186.

Elster N. Less is more: the risks of multiple births. The Institute for Science, Law, and Technology Working Group on Reproductive Technology. *Fertil Steril* 2000;**74**:617–623.

Ericson A, Kallen B, Aberg A. Use of multivitamins and folic acid in early pregnancy and multiple births in Sweden. *Twin Res* 2001;**4**:63–66.

Eriksson A. Variations in the human twinning rate. *Acta Genet* 1962;**12**:242–250.

Eriksson AW, Abbott C, Kostense PJ, Fellman JO. Secular changes of twinning rates in Nordic populations. *Acta Genet Med Gemellol (Roma)* 1995;**44**:141–162.

Eriksson AW, Fellman J. Ethnological differences in twinning trends. *Scand J Clin Lab Invest* 1967;**19**:73.

Eriksson AW, Fellman J. Differences in the Twinning Trends between Finns and Swedes. *Am J Hum Genet* 1973;**25**:141–151.

Eriksson AW, Fellman J. Seasonal variation of livebirths, stillbirths, extramarital births and twin maternities in Switzerland. *Twin Res* 2000;**3**:189–201.

Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. *Lancet* 2005;**365**:1807–1816.

Fellman J, Eriksson AW. Statistical analysis of the seasonal variation in the twinning rate. *Twin Res* 1999;**2**:22–29.

Fryns JP. The female and the fragile X. A study of 144 obligate female carriers. *Am J Med Genet* 1986;**23**:157–169.

Gabler S, Voland E. Fitness of twinning. *Hum Biol* 1994;**66**:699–713.

Galloway SM, McNatty KP, Cambridge LM, Laitinen MP, Juengel JL, Jokiranta TS, McLaren RJ, Luiro K, Dodds KG, Montgomery GW *et al*. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nat Genet* 2000;**25**:279–283.

Gilfillan CP, Robertson DM, Burger HG, Leoni MA, Hurley VA, Martin NG. The control of ovulation in mothers of dizygotic twins. *J Clin Endocrinol Metab* 1996;**81**:1557–1562.

Gosden RG, Treloar SA, Martin NG, Cherkas LF, Spector TD, Faddy MJ, Silber SJ. Prevalence of premature ovarian failure in monozygotic and dizygotic twins. *Hum Reprod* 2007;**22**:610–615.

Greunlich WW. Heredity in human twinning. *Am J Phys Anthrop* 1934;**19**:391–443.

Gromoll J, Simoni M. Follicle-stimulating-hormone receptor and twinning. *Lancet* 2001;**357**:230–232.

Hall JG. Twinning. *Lancet* 2003;**362**:735–743.

Hamamy HA, Ajlouni HK, Ajlouni KM. Familial monozygotic twinning: report of an extended multi-generation family. *Twin Res* 2004;**7**:219–222.

Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R, Galloway SM. Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (Ovis aries). *Biol Reprod* 2004;**70**:900–909.

Healey SC, Duffy DL, Martin NG, Turner G. Is fragile X syndrome a risk factor for dizygotic twinning? *Am J Med Genet* 1997;**72**:245–246.

Helle S, Lummaa V, Jokela J. Selection for increased brood size in historical human populations. *Evolution* 2004;**58**:430–436.

Hunter MG, Robinson RS, Mann GE, Webb R. Endocrine and paracrine control of follicular development and ovulation rate in farm species. *Anim Reprod Sci* 2004;**82–83**:461–477.

Imaizumi Y. Trends of twinning rates in ten countries, 1972–1996. *Acta Genet Med Gemellol (Roma)* 1997;**46**:209–218.

Imaizumi Y. A comparative study of twinning and triplet rates in 17 countries, 1972–1996. *Acta Genet Med Gemellol (Roma)* 1998;**47**: 101–114.

Imaizumi Y. Demographic trends in Japan and Asia. In: Blickstein I, Keith LG, Keith DM (eds). *Multiple Pregnancy*, 2nd edn. London and New York: Taylor and Francis GroupLondon and New York, 2005, 33–38.

James WH. Are 'natural' twinning rates continuing to decline? *Hum Reprod* 1995;**10**:3042–3044.

Jernstrom H, Knutsson M, Olsson H. Temporary increase of FSH levels in healthy, nulliparous, young women after cessation of low-dose oral contraceptive use. *Contraception* 1995;**52**:51–56.

Jewell SE, Yip R. Increasing trends in plural births in the United States. *Obstet Gynecol* 1995;**85**:229–232.

Kallen B. Use of folic acid supplementation and risk for dizygotic twinning. *Early Hum Dev* 2004;**80**:143–151.

Kallen K. Maternal smoking and twinning. *Twin Res* 1998;**1**:206–211.

Kenneson A, Warren ST. The female and the fragile X reviewed. *Semin Reprod Med* 2001;**19**:159–165.

Kiely JL, Kiely M. Epidemiological trends in multiple births in the United States, 1971–1998. *Twin Res* 2001;**4**:131–133.

Kovanci E, Rohozinski J, Simpson JL, Heard MJ, Bishop CE, Carson SA. Growth differentiating factor-9 mutations may be associated with premature ovarian failure. *Fertil Steril* 2007;**87**:143–146.

Krieger H, Colletto GM, Franchi-Pinto C, Beiguelman B. Investigation on seasonality of twin births in Brazil. *Acta Genet Med Gemellol (Roma)* 1996;**45**:397–403.

Laissue P, Christin-Maitre S, Touraine P, Kuttenn F, Ritvos O, Aittomaki K, Bourcigaux N, Jacquesson L, Bouchard P, Frydman R *et al*. Mutations and sequence variants in GDF9 and BMP15 in patients with premature ovarian failure. *Eur J Endocrinol* 2006;**154**: 739–744.

Lambalk CB, Boomsma DI, De Boer L, De Koning CH, Schoute E, Popp-Snijders C, Schoemaker J. Increased levels and pulsatility of follicle-stimulating hormone in mothers of hereditary dizygotic twins. *J Clin Endo Metab* 1998a;**83**:481–486.

Lambalk CB, De Koning CH, Braat DD. The endocrinology of dizygotic twinning in human. *Mol Cell Endo* 1998b;**145**:97–102.

Lambalk CB, Schats R, Bleker OP, Elfering-Stinkens PM, Orlebeke JF. Meerlingzwangerschappen; epidemiologie en beleid. *Ned Tijdschr Geneeskd* 2004;**148**:448–450.

Levy T, Blickstein I. Does the use of folic acid increase the risk of twinning? *Int J Fertil Womens Med* 2006;**51**:130–135.

Lewis CM, Healey SC, Martin NG. Genetic contribution to DZ twinning. *Am J Med Genet* 1996;**61**:237–246.

Li Z, Gindler J, Wang H, Berry RJ, Li S, Correa A, Zheng JC, Erickson JD, Wang Y. Folic acid supplements during early pregnancy and likelihood of multiple births: a population-based cohort study. *Lancet* 2003;**361**:380–384.

Liao WX, Roy AC, Ng SC. Follicle-stimulating-hormone receptor and twinning. *Lancet* 2001;**357**:230–232.

Lieberman J, Borhani NO, Feinleib M. Twinning as a heterozygous advantage for alpha1-antitrypsin deficiency. *Prog Clin Biol Res* 1978;**24**:45–54.

Little J. Descriptive epidemiology. In: MacGillivray I, Campbell DM, Thompson B (eds). *Twinning and twins*. New York: John Wiley and SonsNew York, 1988, 37–66.

Lumley J, Watson L, Watson M, Bower C. Modelling the potential impact of population-wide periconceptional folate/multivitamin supplementation on multiple births. *BJOG* 2001;**108**:937–942.

Lummaa V, Haukioja E, Lemmetyinen R, Pikkola M. Natural selection on human twinning. *Nature* 1998;**394**:533–534.

Macfarlane A, Blondel B. Demographic Trends in Western European Coutries. In: Blickstein I, Keith LG, Keith DM (eds). *Multiple Pregnancy*, 2nd edn. London and New York: Taylor and Francis GroupLondon and New York, 2005, 11–21.

MacGillivray I, Campbell DM. The physical characteristics and adaptations of women with twin pregnancies. *Prog Clin Biol Res* 1978;**24**: 81–86.

MacGillivray I, Samphier M, Little J, Ian M, Dorris MC, Barbara T. Factors affecting twinning. In: MacGillivray I, Campbell DM, Thompson B (eds).*Twinning and twins*, New York: John Wiley and SonsNew York, 1988, 67–97.

Macklon NS, Fauser BC. Regulation of follicle development and novel approaches to ovarian stimulation. *Hum Reprod Update* 2000;**6**:307–312.

Macourt DC, Stewart P, Zaki M. Multiple pregnancy and fetal abnormalities in association with oral contraceptive usage. *Aust NZ J Obstet Gynaecol* 1982;**22**:25–28.

Markovitz J, Hershlag A. Multiple births resulting from assisted reproductive technologies in the united states, 1997–2001. In: Blickstein I, Keith LG, Keith DM (eds). *Multiple Pregnancy*, 2nd edn. London and New York: Taylor and Francis GroupLondon and New York, 2005, 58–67.

Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Munson ML. Births: final data for 2003. *Natl Vital Stat Rep* 2005;**54**:1–116.

Martin JA, Hamilton BE, Ventura SJ, Menacker F, Park MM, Sutton PD. Births: final data for 2001. *Natl Vital Stat Rep* 2002;**51**:1–102.

Martin JA, Park MM. Trends in twin and triplet births: 1980–97. *Natl Vital Stat Rep* 1999;**47**:1–16.

Martin NG, Olsen ME, Theile H, El Beaini JL, Handelsman D, Bhatnagar AS. Pituitary-ovarian function in mothers who have had two sets of dizygotic twins. *Fertil Steril* 1984;**41**:878–880.

Martin NG, Robertson DM, Chenevix-Trench G, de Kretser DM, Osborne J, Burger HG. Elevation of follicular phase inhibin and luteinizing hormone levels in mothers of dizygotic twins suggests nonovarian control of human multiple ovulation. *Fertil Steril* 1991a;**56**:469–474.

Martin NG, Shanley S, Butt K, Osborne J, O'Brien G. Excessive follicular recruitment and growth in mothers of spontaneous dizygotic twins. *Acta Genet Med Gemellol (Roma)* 1991b;**40**:291–301.

Mathews TJ, Hamilton BE. Mean age of mother, 1970–2000. *Natl Vital Stat Rep* 2002;**51**:1–13.

McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. *Endocr Rev* 2000;**21**:200–214.

Meulemans WJ, Lewis CM, Boomsma DI, Derom CA, Van den Berghe H, Orlebeke JF, Vlietinck RF, Derom RM. Genetic modelling of dizygotic twinning in pedigrees of spontaneous dizygotic twins. *Am J Med Genet* 1996;**61**:258–263.

Montgomery GW, Crawford AM, Penty JM, Dodds KG, Ede AJ, Henry HM, Pierson CA, Lord EA, Galloway SM, Schmack AE. The ovine Booroola fecundity gene (FecB) is linked to markers from a region of human chromosome 4q. *Nat Genet* 1993;**4**:410–414.

Montgomery GW, Duffy DL, Hall J, Haddon BR, Kudo M, McGee EA, Palmer JS, Hsueh AJ, Boomsma DI, Martin NG. Dizygotic twinning is not linked to variation at the alpha-inhibin locus on human chromosome 2. *J Clin Endocrinol Metab* 2000;**85**:3391–3395.

Montgomery GW, Duffy DL, Hall J, Kudo M, Martin NG, Hsueh AJ. Mutations in the follicle-stimulating hormone receptor and familial dizygotic twinning. *Lancet* 2001a;**357**:773–774.

Montgomery GW, Galloway SM, Davis GH, McNatty KP. Genes controlling ovulation rate in sheep. *Reproduction* 2001b;**121**:843–852.

Montgomery GW, McNatty KP, Davis GH. Physiology and molecular genetics of mutations that increase ovulation rate in sheep. *Endocr Rev* 1992;**13**:309–328.

Montgomery GW, Scott IC, Johnstone PD. Seasonal changes in ovulation rate in coopworth ewes maintained at different liveweights. *Anim Reprod Sci* 1988;**17**:197–205.

Montgomery GW, Zhao ZZ, Marsh AJ, Mayne R, Treloar SA, James M, Martin NG, Boomsma DI, Duffy DL. A deletion mutation in GDF9 in sisters with spontaneous DZ twins. *Twin Res* 2004;**7**:548–555.

Montgomery GW, Zhao ZZ, Morley KI, Marsh A J, Boomsma DI, Martin NG, Duffy DL. Dizygotic twinning is not associated with methylenetetrahydrofolate reductase haplotypes. *Hum Reprod* 2003;**18**:2460–2464.

Montgomery GW, Zhu G, Hottenga JJ, Duffy DL, Heath AC, Boomsma DI, Martin NG, Visscher PM. HLA and genome wide allele sharing in DZ twins. *Am J Hum Genet* 2006;**79**:1052–1058.

Moore RK, Erickson GF, Shimasaki S. Are BMP-15 and GDF-9 primary determinants of ovulation quota in mammals? *Trends Endocrinol Metab* 2004;**15**:356–361.

Morales-Suarez-Varella MM, Bech BH, Christensen K, Olsen J. Coffee and smoking as risk factors for twin pregnancies. The Danish National Birth Cohort. *Twin Res Hum Genet* 2007;**10**:597–603.

Muggli EE, Halliday JL. Folic acid and risk of twinning: a systematic review of the recent literature, July 1994 to July 2006. *Med J Aust* 2007;**186**:243–248.

Mulsant P, Lecerf F, Fabre S, Schibler L, Monget P, Lanneluc I, Pisselet C, Riquet J, Monniaux D, Callebaut I *et al*. Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. *Proc Natl Acad Sci USA* 2001;**98**:5104–5109.

Murphy MF, Campbell MJ, Bone M. Is there an increased risk of twinning after discontinuation of the oral contraceptive pill? *J Epidemiol Community Health* 1989;**43**:275–279.

Nylander PP. The frequency of twinning in a rural community in Western Nigeria. *Ann Hum Genet* 1969;**33**:41–44.

Nylander PP. Ethnic differences in twinning rates in Nigeria. *J Biosoc Sci* 1971;**3**:151–157.

Nylander PP. Pituitary gonadotropins and multiple births in Nigeria. *Acta Genet Med Gemellol (Roma)* 1974;**22**:198–201.

Nylander PP. Causes of high twinning frequencies in Nigeria. *Prog Clin Bio Res* 1978;**24**:35–43.

Nylander PP. The twinning incidence of Nigeria. *Acta Genet Med Gemellol (Roma)* 1979;**28**:261–263.

Nylander PP. The factors that influence twinning rates. *Acta Genet Med Gemellol (Roma)* 1981;**30**:189–202.

Olsen J, Bonnelykke B, Nielsen J. Tobacco smoking and twinning. *Acta Med Scand* 1988;**224**:491–494.

Palmer JS, Zhao ZZ, Hoekstra C, Hayward NK, Webb PM, Whiteman DC, Martin NG, Boomsma DI, Duffy DL, Montgomery GW. Novel variants in growth differentiation factor 9 in mothers of dizygotic twins. *J Clin Endocrinol Metab* 2006;**91**:4713–4716.

Parazzini F, Chatenoud L, Benzi G, Di Cintio E, Dal Pino D, Tozzi L, Fedele L. Coffee and alcohol intake, smoking and risk of multiple pregnancy. *Hum Reprod* 1996;**11**:2306–2309.

Parisi P, Gatti M, Prinzi G, Caperna G. Familial incidence of twinning. *Nature* 1983;**304**:626–628.

Rothman KJ. Fetal loss, twinning and birth weight after oral-contraceptive use. *N Engl J Med* 1977;**297**:468–471.

Roy A, Matzuk MM. Deconstructing mammalian reproduction: using knockouts to define fertility pathways. *Reproduction* 2006;**131**:207–219.

Schoemaker J, van Weissenbruch MM, Scheele F, van der Meer M. The FSH threshold concept in clinical ovulation induction. *Baillieres Clin Obstet Gynaecol* 1993;**7**:297–308.

Schwartz CE, Dean J, Howard-Peebles PN, Bugge M, Mikkelsen M, Tommerup N, Hull C, Hagerman R, Holden JJ, Stevenson RE. Obstetrical and gynecological complications in fragile X carriers: a multicenter study. *Am J Med Genet* 1994;**51**:400–402.

Sharma K. The twinning rates and epidemiological characteristics of births in southeast Uttar Pradesh, India. *Acta Genet Med Gemellol (Roma)* 1997;**46**:47–56.

Shimasaki S, Moore RK, Otsuka F, Erickson GF. The bone morphogenetic protein system in Mammalian reproduction. *Endocr Rev* 2004;**25**: 72–101.

Souza CJ, MacDougall C, MacDougall C, Campbell BK, McNeilly AS, Baird DT. The Booroola (FecB) phenotype is associated with a mutation in the bone morphogenetic receptor type 1 B (BMPR1B) gene. *J Endocrinol* 2001;**169**:R1–R6.

Taffel SM. Demographic trends in twin births:USA. In: Keith LG, Papiernik E, Keith DM, Luke B (eds). *Multiple pregnancy*, 1st edn. London, UK: The Parthenon Publishing GroupLondon, UK, 1995, 133–143.

Tandberg A, Bjorge T, Bordahl PE, Skjaerven R. Increasing twinning rates in Norway, 1967–2004: the influence of maternal age and assisted reproductive technology (ART). *Acta Obstet Gynecol Scand* 2007;**86**: 833–839.

Terry L. *Vital and Health Statistics United States (Rep. No. 21.1)*. Washington, DC: Public Health Service PublicationWashington, DC, 1962.

Tong S, Caddy D, Short RV. Use of dizygotic to monozygotic twinning ratio as a measure of fertility. *Lancet* 1997;**349**:843–845.

Turner G, Robinson H, Wake S, Martin N. Dizygous twinning and premature menopause in fragile X syndrome. *Lancet* 1994;**344**:1500.

Umstad MP, Lancaster PAL. Multiple Births in Australia. In: Blickstein I, Keith LG, Keith DM (eds). *Multiple Pregnancy*, 2nd edn. London and New York: Taylor and Francis GroupLondon and New York, 2005, 26–32.

Vianna-Morgante AM. Twinning and premature ovarian failure in premutation fragile X carriers. *Am J Med Genet* 1999;**83**:326.

Vollset SE, Gjessing HK, Tandberg A, Ronning T, Irgens LM, Baste V, Nilsen RM, Daltveit AK. Folate supplementation and twin pregnancies. *Epidemiology* 2005;**16**:201–205.

Weinberg W. Beiträge zur Physiologie und Pathologie der Mehrlinggeburten beim Menschen. *Arch Gesamte Physiol* 1901;**88**:346–430.

Weinberg W. Differenzmethode und Geburtenfolge bei Zwillingen. *Genetica* 1934;**16**:282–288.

Welt CK, Smith PC, Taylor AE. Evidence of early ovarian aging in fragile X premutation carriers. *J Clin Endocrinol Metab* 2004;**89**:4569–4574.

White C, Wyshak G. Inheritance in human dizygotic twinning. *N Engl J Med* 1964;**271**:1003–1005.

Wilson T, Wu XY, Juengel JL, Ross IK, Lumsden JM, Lord EA, Dodds KG, Walling GA, McEwan JC, O'Connell AR *et al*. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biol Reprod* 2001;**64**:1225–1235.

Wyshak G, White C. Genealogical study of human twinning. *Am J Public Health Nations Health* 1965;**55**:1586–1593.

Zeleznik AJ. Follicle selection in primates: "many are called but few are chosen". *Biol Reprod* 2001;**65**:655–659.

Submitted on August 14, 2007; resubmitted on September 7, 2007; accepted on October 12, 2007