Timing of Twinning, X-Inactivation and Sex Proportion at Birth

Catherine Derom,[1,2] Robert Vlietinck,[1,2] and Robert Derom[2]

[1] *Department of Human Genetics, University Hospital Gasthuisberg, Katholieke Universiteit Leuven, Leuven, Belgium*
[2] *Association for Scientific Research in Multiple Births, Ghent, Belgium*

Twins are not a homogeneous group. According to zygosity and chorionicity essential differences exist. The lower sex proportion at birth is due to the monozygotic twins, especially the monoamnionic variety. X-inactivation patterns in monozygotic twin girls are totally symmetrical in monoamnionic pairs, almost symmetrical in monochorionic diamnionic pairs and can be very asymmetrical in the dichorionic variety.

The East Flanders Prospective Twin Survey (EFPTS), established in 1964 in the Province of East Flanders, Belgium, registers all multiple births within the province. The EFPTS is the only large register that includes placental data and allows differentiation of the three subtypes of monozygotic (MZ) twins as based on the time of the zygotic division: the dichorionic-diamnionic pairs (early, before the 4th day post fertilization), the monochorionic-diamnionic pairs (intermediate, between the 4th and the 7th day post fertilization) and the monochorionic-monoamnionic pairs (late, after the 8th day post fertilization). This prompted us to study the consequences of early embryological events (before and just after implantation of the embryo) on two important biological phenomena's: X-inactivation and sex proportion at birth.

Sex Proportion at Birth

The proportion of males in multiple births has been and still is a matter of lively debate (James, 1980). Clearly, there are differences between singleton and multiple births. The proportion of males in the human species decreases with each increase in the number of fetuses per pregnancy (Nichols, 1952). According to our data from East Flanders (see Table 1) and data from the literature (James, 1986), it may be concluded that the sex proportion in MZ twins is lowered, albeit to a smaller extent in the dichorionic and monochorionic diamnionic and a much greater extent in the monoamnionic variety (Derom et al., 1988).

One can only speculate about the reasons for this remarkable biological phenomenon. Various hypotheses have been put forward, including those of Guerrero (1970) and James (1971) who independently suggest that the sex of a human zygote is influenced by the time of its formation within the menstrual cycle, with more male zygotes being formed both early and very late in the cycle, whereas more female zygotes are formed during the middle of the cycle. Experiments in lower vertebrates including fish, amphibians and chickens lead (James, 1971) to MZ twinning by retarding the development of the fertilized ova, depriving them of oxygen, or maintaining them at a lower temperature. Delayed ovulation seems to induce MZ twinning in rabbits (Bomsel-Helmrich & Papiernik, 1976). As suggested by James, it seems possible that the delay hypothesized as being associated with the formation of female zygotes runs parallel with the delay associated with the splitting of the ovum (James, 1971). If this is the case, then MZ twins could be composed of a higher proportion of females.

One or two per cent of MZ pairs of twins share the chorion and the amnion at birth. They represent twinning that occurred after the differentiation of the amnion (about day 7). Incomplete splitting of the embryo is generally considered as giving rise to conjoined twins and could then occur even later, after the second week of development. However, we favor the more probable partial-fusion hypothesis (Spencer, 2003) according to which no difference in time of splitting has to be necessarily considered. Because of the rarity of conjoined twins, it is as yet unknown whether further subdivision of the monoamnionic twins in unjoined and conjoined pairs will clarify the enigma of

Table 1
Sex Proportion of Spontaneous Twins in EFPTS (1964–2004) According to Zygosity and Placentation

Zygosity and chorionicity	Sex proportion
Dizygotic	0.52
Monozygotic–dichorionic	0.50
Monochorionic–diamnionic	0.50
Monochorionic–monoamnionic	0.22
All monozygotic	0.49
All (n = 4942 pairs)	0.51

https://doi.org/10.1375/twin.10.supp.8 Published online by Cambridge University Press

their very low sex proportion. As only three conjoined pairs (all of them female) emerged in our relatively large series, only a registry of almost continental scale could provide the amount of data needed to answer this question. It is well documented that conjoined twins include a high proportion of girls (James, 1980).

It is useful to inquire if female embryos are more likely to undergo delayed splitting than male embryos or if embryonal or fetal mortality in the late-splitting group predominantly affects the male embryos. In their series of spontaneously aborted twins, Uchida et al. (1983) found two male conjoined pairs with normal chromosomes, representing two consecutive abortions in the same mother. Because both conjoined twins and male monoamnionic twins are rare, this single finding could suggest a predominantly male early fetal mortality in monoamnionic twins. With regard to the first question, Burn et al. (1986) hypothesized that unequal lyonization may represent a cause of late twinning unique to female embryos. If this is true, analysis of the DNA methylation patterns of the X chromosome in monoamnionic twins should shed more light on the question. This hypothesis has been demonstrated to be non probable, however, by studying X-inactivation in MZ female pairs whereby X-inactivation is totally symmetrical in monochorionic–monoamnionic pairs, almost symmetrical in monochorionic–diamnionic pairs and asymmetrical in dichorionic–MZ pairs (Chitnis et al., 1999; Monteiro et al., 1998).

Another more plausible explanation for the very low sex proportion in the monoamionic twin group is the relative delay in early development of female embryos (Loos et al., 2001; Pergamente al., 1994). As a result, female embryos could be somewhat less mature at the time of formation of the amnion, and splitting of female embryos may be more compatible with survival at this stage. The delay in early female development has been ascribed to the absence of the Y chromosome (Pergament et al., 1994). However, the process of X-inactivation, since it may occur when there are less then 10 cells in the embryo (Puck, 1998), might itself contribute to a slight delay in early female development.

The proportion of same-sex and opposite-sex pairs found in dizygotic (DZ) twins is in accordance with the Weinberg rule as reported earlier by our group (Vlietinck et al., 1988). However, our data do not rule out subtle secular changes in that proportion as suggested by James (1992) and Orlebeke et al. (1991) This discussion is not purely academic, because the Weinberg rule, although it has been criticized frequently, is the major tool available for the study of trends in MZ and DZ twinning rates when zygosity is not known.

The sex proportion discussed so far deals with spontaneous twinning, rather than iatrogenic twins. A series of studies have addressed this question, but the results are not in agreement. No large-scale population-based enquiries have been performed. Hence, one can question whether the samples are representative. In the EFPTS, the sex proportion of the iatrogenic twins does not differ significantly from that of the spontaneous twins: 0.513 (95% confidence interval [CI] 0.533–0.493) for iatrogenic DZ and 0.52 (95%CI 0.534-0.507) for spontaneous DZ twins. This does not support James' hypothesis (1986) that hormonal induction of ovulation increases the mother's gonadotropin levels at the time of conception and that this in turn increases the probability of male offspring.

X-Inactivation

MZ twinning events appear to occur very soon after fertilization, presumably spanning a time frame of as much as a week or more after conception (Bulmer, 1970). The estimates for the timing of splitting of the embryo are based primarily on the examination of the placental anatomy of MZ twins at birth. Thus, it has been postulated that dichorionic MZ twinning occurs early, before blastocyst formation, whereas monochorionic MZ twinning occurs later. To gain insight into the timing of twinning, we have examined a closely related event, X-chromosome inactivation. Because of X-inactivation, tissues of females are normally a mosaic of two cell populations, each expressing gene alleles from either the paternal or the maternal X chromosome. This process of X-inactivation has been estimated to take place within the time frame at which MZ twinning is thought to occur (Puck, 1998).

X-chromosome inactivation patterns were investigated in the blood and buccal mucosa from 33 MZ and 5 DZ female twin pairs. Whereas X-inactivation patterns in peripheral blood, which is mesodermal in origin, may differ among the members of a MZ twin pair, we reported that this phenomenon is restricted to those twins with a dichorionic anatomy. Monochorionics do not differ substantially in their patterns of X-inactivation. The generally accepted explanation for this finding is that monochorionic twins share their placental blood supply during intrauterine life, whereas dichorionic MZ twins do not and therefore similar X-inactivation patterns in this shared hemopoietic cell population would be expected in monochorionic twin pairs (Leroy, 1991). We therefore investigated X-inactivation patterns in the buccal mucosa of MZ twin girls, a tissue of ectodermal origin which is presumably not exchanged during intrauterine development. Again monochorionic MZ showed highly similar X-inactivation skewing (< 6% difference between one twin to the other) while the dichorionic MZ twins had significantly greater mean differences in skewing (14%; see figure; Monteiro et al., 1998).

These data demonstrate that monochorionic MZ twins have strikingly correlated X-inactivation patterns in both ectodermal and mesodermal tissues, whereas dichorionic MZ twins do not. Monochorionic MZ twins are thought to result from a later twinning event than dichorionic MZ twins (Leroy, 1991). Therefore, it is proposed that the timing of commitment to X-inactivation occurs between the time of dichorionic and monochorionic MZ twinning, possibly around 4 days

https://doi.org/10.1375/twin.10.supp.8 Published online by Cambridge University Press

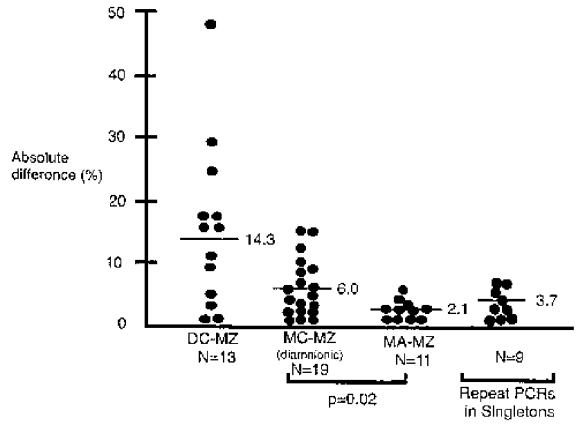

Figure 1

Summary of differences in X-inactivation patterns among various subtypes of MZ twin pairs and among repeat assays of singletons. An HpaII/PCR methylation assay for the androgen-receptor gene was performed on buccal mucosal DNA, as described elsewhere (Chitnis et al., 1999; Monteiro et al., 1998).

Note: DC: dichorionic; MC: monochorionic; MA: monoamniotic; MZ: monozygotic

after conception (Monteiro et al., 1998). If this hypothesis is correct, the similarity in X-inactivation of monochorionic MZ pairs would be explained by the fact that splitting occurred after commitment to X-inactivation, with both embryos deriving from a cell population in which X-inactivation patterns were already established.

These conclusions were further confirmed by studying X-inactivation patterns in buccal mucosa of monoamnionic twin girls. Monoamnionic MZ twinning is a relatively rare event and thought to result from a very late splitting of the human embryo (after day 7 to 8 post fertilization). As hypothesized, the monoamnionic-MZ twins exhibit identical X-inactivation patterns (see Figure; Chitnis et al., 1999).

References

Bomsel-Helmrich, O., & Papiernik, E. (1976). Delayed ovulation and monozygotic twinning. *Acta Geneticae Medicae et Gemellologiae, 25,* 73–76.

Bulmer, M. C. (1970). *The biology of twinning*. London: Oxford University Press.

Burn, J., Povey, S., & Boyd, Y. (1986). Duchenne muscular dystrophy in one of monozygotic twin girls. *Journal of Medical Genetics, 23,* 494–500C

Chitnis, S., Derom, C., Vlietinck, R., Derom, R., Monteiro, J., & Gregerson, P. (1999). X-chromosome-inactivation patterns confirm the late timing of monoamniotic-MZ twinning. *American Journal of Human Genetics, 65,* 570–571.

Derom, C., Vlietinck, R., Derom, R., Van den Berghe, H., & Thiery, M. (1988). Population-based study of sex proportion in monoamniotic twins. *New England Journal of Medicine, 319,* 119–120.

Guerrero, R. (1970). Sex ratio: A statistical association with the type and time of insemination in the menstrual cycle. *International Journal of Fertility, 15,* 221–225.

James, W. H. (1971). Cycle day of insemination, coitus rate and sex proportion. *Lancet, 1,* 112.

James, W. H. (1980). Sex ratio and placentation in twins. *Annals of Human Biology, 7,* 273–276.

James, W. H. (1986). Hormonal control of sex ratio. *Journal of Theoretical Biology, 118,* 427–441.

James, W. H. (1992). The current status of Weinberg's differential rule. *Acta Geneticae Medicae et Gemellologiae, 41,* 33–42.

Leroy, F. (1991). Early embryology and placentation of human twins. In R. Dulbecco (Ed.), *Encyclopedia of Human Biology* (pp. 305–315). New York: Academic Press.

Loos, R. J. F., Derom, C., Eeckels, R., Derom, R., & Vlietinck, R. (2001). Length of gestation and birth weight in dizygotic twins. *Lancet, 358,* 560–561.

Monteiro, V., Derom, C., Vlietinck, R., Kohn, N., Lesser, M., & Gregerson, P. K. (1998). Commitment to X-inactivation precedes the twinning event in monochorionic MZ twins. *American Journal of Human Genetics, 63,* 339–346.

Nichols, J. B. (1952). Statistics of births in the United States. *American Journal of Obstetrics and Gynecology, 64,* 376–381.

Orlebeke, J., Eriksson, A. W., Boomsma, D. I., Vlietinck, R., Tas, F. J., & de Geus, E. C. (1991). Changes in the DZ unlike/like sex ratio in the Netherlands. *Acta Geneticae Medicae et Gemellologiae, 40,* 319–323.

Pergament, E., Fiddler, M., Cho, N., Johnson, D., & Holmgren, W. J. (1994). Sexual differentiation and preimplantation cell growth. *Human Reproduction, 9,* 1730–1732.

Puck, J. M. (1998). The timing of twinning: More insights from X-inactivation. *American Journal of Human Genetics, 63,* 327–328.

Spencer, R. (2003). *Conjoined twins. Developmental malformations and clinical implications*. Baltimore, MD: John Hopkins University Press.

Uchida, I. A., Freeman, V. C., & Gedeon, M. (1983). Twinning rate in spontaneous abortion. *American Journal of Human Genetics, 35,* 987–993.

Vlietinck, R., Papiernik, E., Derom, C., Grandjean, H., Thiery, M., & Derom, R. (1988). The European Multiple Birth Study (EMBS). *Acta Geneticae Medicae et Gemellologiae, 37,* 27–30.

https://doi.org/10.1375/twin.10.supp.8 Published online by Cambridge University Press