maximum period, and a substantial number of patients completed the two cycles within a much shorter period. Furthermore, they are suggesting that waiting for the FSH level to drop will result in a better outcome but—again—do not provide any evidence for this assertion. It would have been more valuable if they were able to provide a comparison between their patients who had a persistently raised FSH level and decided to proceed with treatment despite being 'extensively counselled' and those in whom the FSH had dropped before starting treatment.

It is reasonable to defer the start of treatment cycles pending completion of interventions with proven benefit to IVF outcome such as surgical treatment of hydrosalpinges. We are simply questioning whether there is any evidence that deferring treatment while repeatedly testing FSH levels improves outcome.

We believe that if the FSH level is elevated, the clinician should help the patient accept the situation and discuss the options of starting treatment or explore alternative options such as egg donation. The issue therefore is this: Should both clinician and patient accept a lower pregnancy rate for these patients? Or should they continue to hunt for a target FSH level with no proven benefit? We believe that the latter approach wastes valuable time for patients, with the ultimate result that some may give up altogether.

Reference

Abdalla H and Thum MY (2006) Repeated testing of basal FSH levels has no predictive value for IVF outcome in women with elevated basal FSH. Hum Reprod 21(1),171–174.

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doi:10.1093/humrep/del244

Mosaic/chimeras and twinning in the current reproductive genetics perspective

Sir,

I read with great interest the article by Charles Boklage (Boklage, 2006) discussing the origin of twin-associated spontaneous chimeras. The author vividly reviews current controversies in this problem and envisages his non-canonical observations and inferences. The twinning is an essential part of human reproduction biology. It has an important genetical and medical implications greatly increased because of the assisted reproduction techniques (ART) era. Monozygotic (MZ) twins are viewed as a natural genetic engineering and cloning event. Comparison of MZ and dizygotic (DZ) twins remains one of the most fruitful approaches in the human genetics. However, understanding of twinning mechanisms, as Boklage concludes, need to be re-examined in the light of current discoveries in human reproductive biology and genetics. The author mentions predominant paternal origin of triploids, paternal centrosome inheritance, paternally-dependent family twinning, placental chimerism/mosaism and 'vanishing twins', whole body chimerism and maternal long-lasting microchimerism.

From this perspective, the author challenges deeply rooted outlooks and common knowledge about the pattern of MZ/DZ twinning and origins of chimeras. He suggests that most chimeras like other DZ are born single and arise from mono-ovular embryos. In his opinion, an embryo commitment to twinning occurs before the differentiation of the chorion because of an anterior midline asymmetry. The author cites his original statistical data on similarity of DZ and MZ in relation to handedness and some symmetry features as being the necessary consequences of events inside a single mass of cells. He stresses that recent findings of monochorionic DZ twins strongly confirms his alternative model.

I find very important the main conclusion that 'human spontaneous chimerism is common', even if one disagrees with an indicated high spontaneous chimerism rate ~10% of all population. It is relevant to remember the previous similar inference by Finnish cytogeneticists who found the translocation marked tetragametic chimera: 'chimerism is considered to be a rarity in man, but it is possible that known cases represent only a small portion of the true incidence, since clinically normal chimeric individual are not discovered' (Nyberg *et al*., 1992). The IVF data confirm and strengthen this prediction (Bonthron, 2004).

I would like to make some critical remarks and additions. In the heat of the argument, the author was inclined to deny the principal differences in the origin and genetic status of the two kinds of twins. However, cited statistical data on similarity of DZ and MZ twins in relation to handedness and some symmetry-depending features cannot abandon the general rightness of the firmly established principal differences of the DZ–MZ twins (Leroy, 1991; Hall, 2003). The same concerns his critics of the classical Weinberg approach for the rough estimation of DZ : MZ population ratio that is used as a reasonable measure of human fertility (Tong and Short, 1998). The situation seems in principal similar with Mendel's laws. Their application in practice has definite constrains and limitations, but even deviations from the famous ratios 3:1 or 9:3:3:1 do not shake their general solid genetic/chromosomal basis. Certainly, we need the direct embryogenetic data of an ultrasound noting of corpora lutea number, chorionicity and zygosity of appearing twins (Tong *et al*., 2004).

At the same time, I would like to support the author's emphasis on the fertilization errors connected with equal secondary oocyte division and double fertilization as the main source of resulting mono-ovular twin/chimeras. The potential repertoire and real implementation of such events leading to chimerism in mammals were analysed in the influential classical book of McLaren (1976). Now the analysis has reached humans. The fluorescence in-situ hybridization (FISH) and microsatellite DNA techniques have shown an existence in humans of unusual cases of chimerism/mosaicism and twinning. For my knowledge, the best relevant example is the parthenogenetic/normal male chimera eloquently analysed by Bonthron and co-authors (Strain *et al*., 1995). The boy had identical gynogenetic Xm1/Xm1 blood cells with almost entirely normal Xm1/Y fibroblasts. He appeared neither a

mosaic nor a chimera. Both his cell lines originated from the same oocyte but not derived from a common zygote. The origin of such a chimera needs simultaneous implementation of at least five reproductive errors involving the *heterochrony* and *general zygotic instability*: (i) oocyte parthenogenetic activation before fertilization, (ii) sperm penetration in blastomere because of delayed polyspermy block, (iii) delayed sperm entry in the first mitosis, (iv) diploidization of the second haploid blastomere and (v) constitution and survival of parthenogenetic chimera.

Before this chimera was described, most reproductive biologists and experienced clinicians would have thought it unbelievable. Surani (1995) concluded that spontaneous parthenogenetic activation and cleavage/blastogenesis may be relatively common in humans but remain undetected because of the imbalance and early abortions. Similar heterochrony/instability errors were suggested for the origin of dispermic trigenomic chimera, including two paternal and one maternal genomes (Giltay *et al*., 1998).

Such chimeras have quirk asymmetric phenotypic deviations as in similar mouse chimeras where parthenogenetic cells contribute to brain in higher numbers than to other tissues. Accordingly, the observed left-sided microsomia of the chimeric boy may have resulted from retarded growth of tissues with parthenogenetic cell lineage (Strain *et al*., 1995). Can we expect a 'schizophrenia predisposition' in such body chimeric schizm?

The third reproductive abnormality (in addition to heterochrony and general zygotic instability) leading to unusual chimera/mosaic and twin associations is the postzygotic diploidization of triploids. This phenomenon was first observed by Angell *et al*. (1986) and then confirmed by many reproductive biologists (see review Golubovsky, 2003). Up to 6% of human oocytes are penetrated by two spermia resulting in the appearance of tripronuclear [three pronuclei (3PN)] zygotes. The cleavage divisions of diandric 3PN zygotes are characterized by peculiar types of genome instability: immediate exclusion of one or two whole set of genomes and a higher incidence of aneuploid cells because of tripolar spindles.

Recently, this immediate postzygotic dipolidization of 3 PN zygotes was analysed by FISH technique including chromosome 4, 13, 18, 21, X and Y (Pang *et al*., 2005). After the first cleavage, >20% of blastomeres appeared pure 2n and >50% appeared genome mosaics, like 2n/3n and 1n/2n. It is expected that these 1n blastomeres will follow to endocytosis with resulting haploid diploidization (Strain *et al*., 1995; Surani, 1995). 3PN zygotes may produce pure 3n and 2n/3n mosaics, pure 2n bipaternal and 2n androgenetic derivatives (hydatidiform moles). Increased incidence both triploidy and twinning in some families was found in the first comprehensive cytological studies on human triploidy (Uchida and Freeman, 1985). In cell/tissue progeny of 3PN zygotes, there are expected an unusual mono-ovular chimera/mosaics and diverse twin/mole oddities (Golubovsky, 2003). Any discussed chimera/twin landscape must definitely include diverse chimeric associations comprising triploid partial and diploid complete moles (regretfully, they are not mentioned in Boklage's article). There were described unusual fetus/mole associations of a genotype Xm1Xp1/Xp1Xp1, with identical paternal genome and presumed mono-ovular origin (Makrydimas *et al*., 2002). The authors suggest precocious (heterochronous) male PN mitotic division leading to triploid zygote Xp1Xp1Xm. This triploid after elimination of one Xp1 genome and its successive endocytosis may give two 2n derivatives, normal Xm1Xp1 fetus and complete Xp1Xp1 homozygous mole. This 'triploid scenario' of 2n mole formation is natural and does not need the fertilization of a mythical 'empty eggs', which were never observed or described in 10s of 1000s of IVF cases. Another similar recent chimera finding—twin pregnancy with a chimeric androgenic (Xp1/Xp1 + Xm1/Y) and biparental placenta (Surti *et al*., 2005) might be mono-ovular derivatives of Xm1Xp1Y triploid zygote that gave diploid mole/fetus Xm1/Y and Xp1Xp1 cleavage lineages.

I completely agree with Boklage's inference that the recently found monochorionic dizygotic twins (Redline, 2003; Souter *et al*., 2003) provide an unavoidable lessons of existence of mono-ovular twin zygotes, in a single zona pellucida forming one chimeric entity or chimeric twins. There are a lot of genetic and indirect cytogenetic data that dispermy and occurrence of two male PN in one zygote may lead to an unusual mono-ovular twins and chimeras. Resulting twins on their genetic ontology are expected intermediate between MZ and DZ. The possibility of the 'third type' of twins was mentioned in a comprehensive review (Leroy, 1991). Expected mono-ovular unusual twins have been coined as sesquizygotic (SZ) twins (Golubovsky, 2002). The distinct SZ twin oddities intermediate between typical MZ and typical DZ are depicted in Figure 1. Variant A at left corresponds to MZ twins who have an identical genomes but may be dissimilar on gene/DNA and phenotype/genotype features including epigenotypic ones (Boomsma *et al*., 2002; Hall, 2003). The variant E corresponds to typical DZ biovular twins having distinct maternal and paternal genomes. Variants B, C and D in the figure designate diverse types of SZ twins originating because of paternal fertilization errors—heterochrony, diplospermy and dispermy. Their genetic status is intermediate between MZ and DZ. Heterochronous scenario 'B' suggests precocious division of male PN with the resulting Xm1–1/Xp1 and Xm1–2/Xp1 twins having identical paternal but different M1 recombinant maternal genomes. Variant C pictures diplospermy scenario and variant D—dispermy. Evidently, dispermic SZ twins are hard in practice to discriminate from usual DZ twins. Suffice it to say that an analysis of one pair of MCDZ twins was made by international group of 11 scientists (Souter *et al*., 2003).

However, the situation is not so hopeless. The families with long-lasting hereditary paternal twinning (StClair and Golubovsky, 2002) may provide the natural selective system for search of unusual cases of primary chimeras and SZ twins. Their real population incidence remains unknown, but at least we have started to imagine much better the possible reproduction situations where the search might be fruitful (Golubovsky, 2002, 2003).

Acknowledgement

I would like to thank Julia Goluboskaya for the remarkable Figure design.

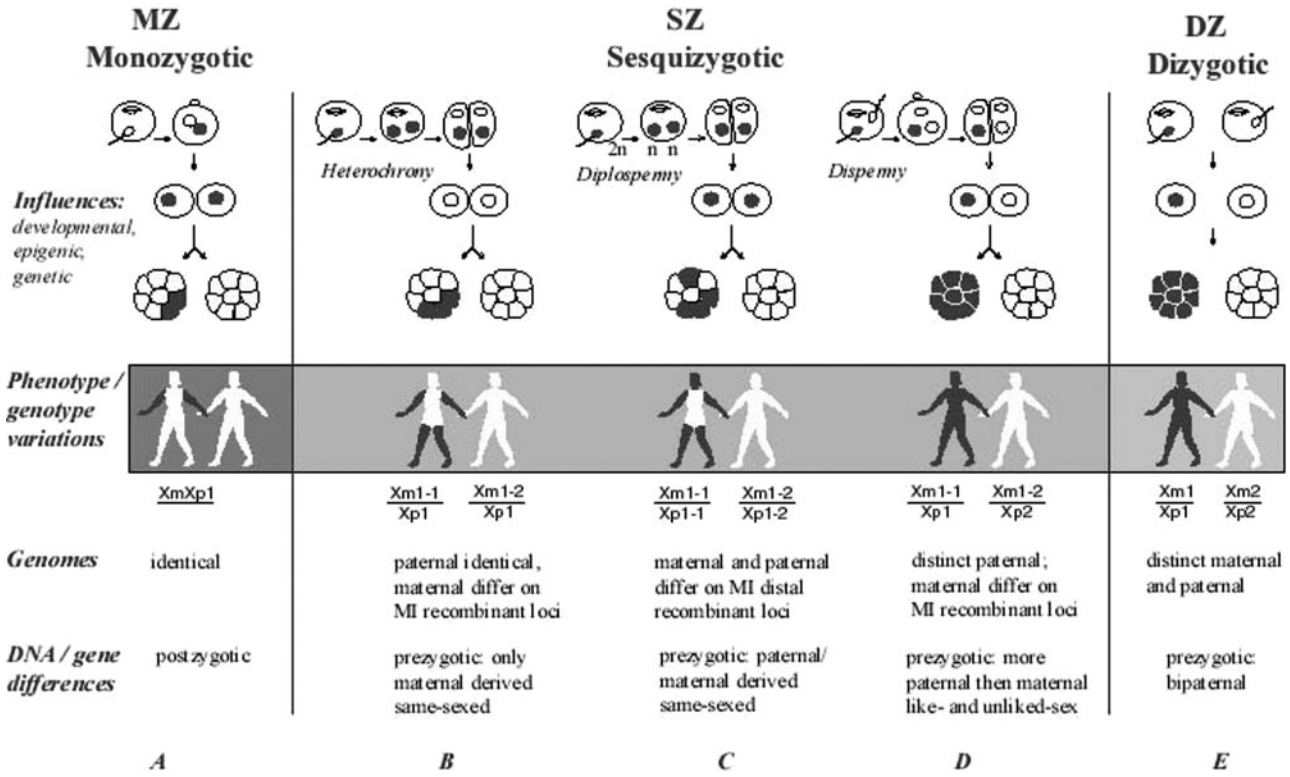

Figure 1. The diverse possible kinds of non-canonical sesquizygotic (SZ) twins intermediate between usual MZ and DZ twins. The SZ twins may appear as mono-ovular entities because of various paternal-dependent fertilization errors like heterochrony, diplospermy and dispermy. They will differ on their genetic status and genotype/phenotype differences indicated at left and depicted schematically on the appropriate figures of twins. It is hard to discriminate in practice the dispermic mono-ovular SZ twins and usual DZ twins. Instead of SZ twins the same scenarios may lead to occurrence of the mono-ovular chimeras, differing on the level of their genetic differences.

References

Angell RB, Templeton AA and Messinis IE (1986) Consequencies of polyploidy in man. Cytogenet Cell Genet 42,1–7.

Boklage CE (2006) Embryogenesis of chimeras, twins and anterior midline asymmetries. Hum Reprod 21,579–591.

Bonthron DT (2004) XX/XY chimerism after IVF. Prenat Diagn 24,573–580.

Boomsma D, Busjahn A and Peltonen L (2002) Classical twin studies and beyond. Nat Genet 3,872–882.

Giltay JC, Brunt T, Beemer FA, Wit JM, Amstell HK, Pearson PL and Wijmenga C (1998) Polymorphic detection of a parthenogenetic maternal and double paternal contribution to a 46,XX/46XY hermaphrodite. Am J Hum Genet 62,937–940.

Golubovsky MD (2002) Paternal familial twinning: hypothesis and genetic/medical implications. Twin Res 5,75–78.

Golubovsky MD (2003) Postzygotic diploidization in triploids as a source of unusual cases of mosaicism and twinning. Hum Reprod 18,236–242.

Hall G (2003) Twinning. Lancet 362,735–743.

Leroy F (1991) Embryology and placentation in twins. In *Encyclopedia of Human Biology*, Vol. 3. Academic Press, San Diego, pp. 305–315.

McLaren A (1976) *Mammalian Chimeras*. Cambridge University Press, Cambridge.

Makrydimas G, Sevire NJ, Thornton SE, Zagorianakou N, Lolis D and Fisher RA (2002) Complete hydatidiform mole and normal live birth: a novel case of confined placental mosaicism. Hum Reprod 17,2459–2463.

Nyberg RH, Haapala AK and Simola KO (1992) A case of human chimerism detected by unbalanced chromosomal translocation. Clin Genet 42,257–259.

Pang MG, Jee BC, Kim SH, Ryu BY, Oh SK, Suh CS and Moon SY (2005) Chromosomal constitution of embryos derived from tripronuclear zygotes studied by fluorescence in situ hybridization using probes for chromosomes 4, 13, 18,21, X and Y. Gynecol Obstet Invest 59,14–18.

Redline WR (2003) Nonidentical twins with a single placenta-disproving dogma in perinatal pathology. N Engl J Med 349,111–114.

Souter VL, Kapur RP, Nyholt DR, Skogerboe K, Myerson D, Ton CC, Opheim KE, Easterling TR, Shields LE, Montgomery GW and Glass IA. (2003) A report of dizygous monochorionic twins. N Engl J Med 349,154–158.

StClair JB and Golubovsky MD (2002) Paternally derived twinning: a two century examination of records of one Scottish name. Twin Res 5,294–307.

Strain L, Warner JP, Johnstom T, Bonthron DT (1995) A human parthenogenetic chimera. Nat Genet 11,111–113.

Surani MA (1995) Parthenogenesis in man. Nature Genet 11,111–113.

Surti U, Hill LM, Dunn J, Prosen T and Hoffner L (2005) Twin pregnancy with a chimeric androgenic and biparental placenta in one twin displaying mesenchymal dyspalsia phenotype. Prenat Diagn 25,1048–1956.

Tong S and Short RV (1998) Dizygotic twinning as a measure of human fertility. Human Reprod 13,95–98.

Tong S, Vollenhoven B and Meagher S (2004) Determining zygosity in early pregnancy by ultrasound. Ultrasound Obstet Gynecol 23,36–37.

Uchida IA and Freeman VC (1985) Triploidy and chromosomes. Am J Obstet Gynecol 151,65–69.

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doi:10.1093/humrep/del294