The importance of zygosity knowledge for twins, parents and professionals

Jeffrey M. Craig, Murdoch Children's Research Institute, Royal Children's Hospital and Department of Paediatrics, University of Melbourne

Tessa L. Cutler, Twins Research Australia, Melbourne School of Population and Global Health, University of Melbourne, Australia

Kate Murphy, Twins Research Australia, Melbourne School of Population and Global Health, University of Melbourne, Australia

Monica Rankin, Chairperson, International Council of Multiple Birth Organisations, Australia

Jane Denton, Director, Multiple Births Foundation, Queen Charlotte's & Chelsea Hospital, London, UK

Deborah Schofield, Professor and Chair of Health Economics, Faculty of Pharmacy, Boden Institute of the University of Sydney, Murdoch Children's Research Institute, Garvan Institute of Medical Research, Australia

Margaret Otlowski, Dean & Head of School, Faculty of Law, University of Tasmania, Australia

Mark P. Umstad, Director of Maternity Services, Royal Women's Hospital, Melbourne, University of Melbourne Department of Obstetrics and Gynaecology, Victoria, Australia

Susan E. Carrick, The Charles Perkins Centre's Twin Research Node, University Of Sydney, Australia

Zygosity refers to the degree of genetic similarity within each pair of twins. Monozygotic (identical) twins have the same genetic sequence, whereas dizygotic twins differ genetically as much as any pair of siblings. Accurate knowledge of zygosity is important for a range of reasons; yet incorrect information on whether twins are monozygotic or dizygotic is still given to twins and their parents. This article focuses on why zygosity testing is so important medically, socially, financially and ethically.
Keywords: twins, zygosity, ethics, medicine, social issues, parenting

Monozygotic (MZ) twins result from the splitting of a single embryo early in gestation and are genetically the same (McNamara et al., 2016; Hall, 2003). Dizygotic (DZ) twins result from two separate fertilisation events and are as genetically similar as non-twin siblings. In some cases, zygosity can be determined from information about the sex and chorionicity (number of placentas - see below for more details) of twins. In other cases, only a genetic test for zygosity can provide accurate information. The same applies to higher order multiple births. Hence, in this paper, for ease of reading, the word 'twins' refers to all multiples.

Our research (Cutler et al., 2015) and that of others (van Jaarsveld et al., 2012; Bamforth & Machin, 2004) has found that a substantial proportion of parents and twins are misinformed about zygosity status; twins may be misinformed by their parents, and twins and parents may be misinformed by medical professionals. Misinformation is often based on incorrect assumptions such as the belief that only DZ twins can have two placentas/chorions, and that twins can only be MZ if they are physically and behaviourally identical.

The determination of zygosity at birth of all same-sex twins has been recommended by geneticists and epidemiologists since the early 1990s (Craig et al., 2015; Machin, 2004; Bajoria & Kingdom, 1997; Derom et al., 1991). This recommendation was made because the information is useful in determining organ transplant compatibility, for research concerning the biology and pathology of MZ twinning, and for the investigation of many genetic diseases and disorders with multifactorial inheritance. Knowledge of zygosity also provides twins and their families with understanding of their identity and relationships.

TWIN TYPES

There are two main types of twins (See Figure 1). Dizygotic twins result from two separate fertilisation events. Approximately half are the same sex and half are of different sex. Each twin has its own placenta which is continuous with the chorion (outer membrane) and its own amnion (inner sac) with rare exceptions, and each pair are as genetically similar as any siblings. Monozygotic twins result from the splitting of a single embryo early in gestation. Our current understanding of MZ twins indicates that approximately one-third have separate placentas and approximately two-thirds share a single placenta despite maintaining their own amnion, umbilical cord and share of the placental mass. All MZ twins are the same sex with rare exceptions and are genetically 100% identical with rare exceptions. Sharing a placenta results in vascular (blood vessel) connections in all monochorionic twin pairs, with one fifth of these exchanging significant amounts of blood which may cause injury and possibly death to both twins if left untreated.

λ

Lamda

DETERMINING ZYGOSITY

Three parameters are used together to differentiate between MZ and DZ twins: chorionicity, sex and genetic zygosity testing (See Figure 2) (Machin, 2009; Hall, 2003; Segal, 1984). Blood type may also be useful as differing blood types indicate dizygosity. Chorionicity is most accurately determined by the thickness of the membrane between the twins, most accurately assessed before 14 weeks gestation (See Figure 3) (Maruotti et al., 2016). In dichorionic twins a thick membrane separating the twins, forms a lambda shape.

In monochorionic twins this membrane is much thinner and joins the placenta to

Figure 1. The formation of the main types of twins

DZ twins result from two separate fertilisation events. Each has its own placenta which is continuous with the chorion and its own amnion (inner sac) and they are referred to as 'dichorionic diamniotic'. MZ twins result from the splitting of a single embryo early in gestation; the timing of splitting determines the type of MZ twin. Approximately one-third of MZ twins split early and are dichorionic diamniotic. Approximately two-thirds of MZ twins split later and share a single placenta despite maintaining their own amnion, umbilical cord and share of the placental mass. They are referred to as 'monochorionic diamniotic'. Approximately 1% of twins split even later and share a single chorion and amnion. They are referred to as 'monochorionic monoamniotic' or 'MoMo'. (Taken from McNamara et al., 2016, with permission)

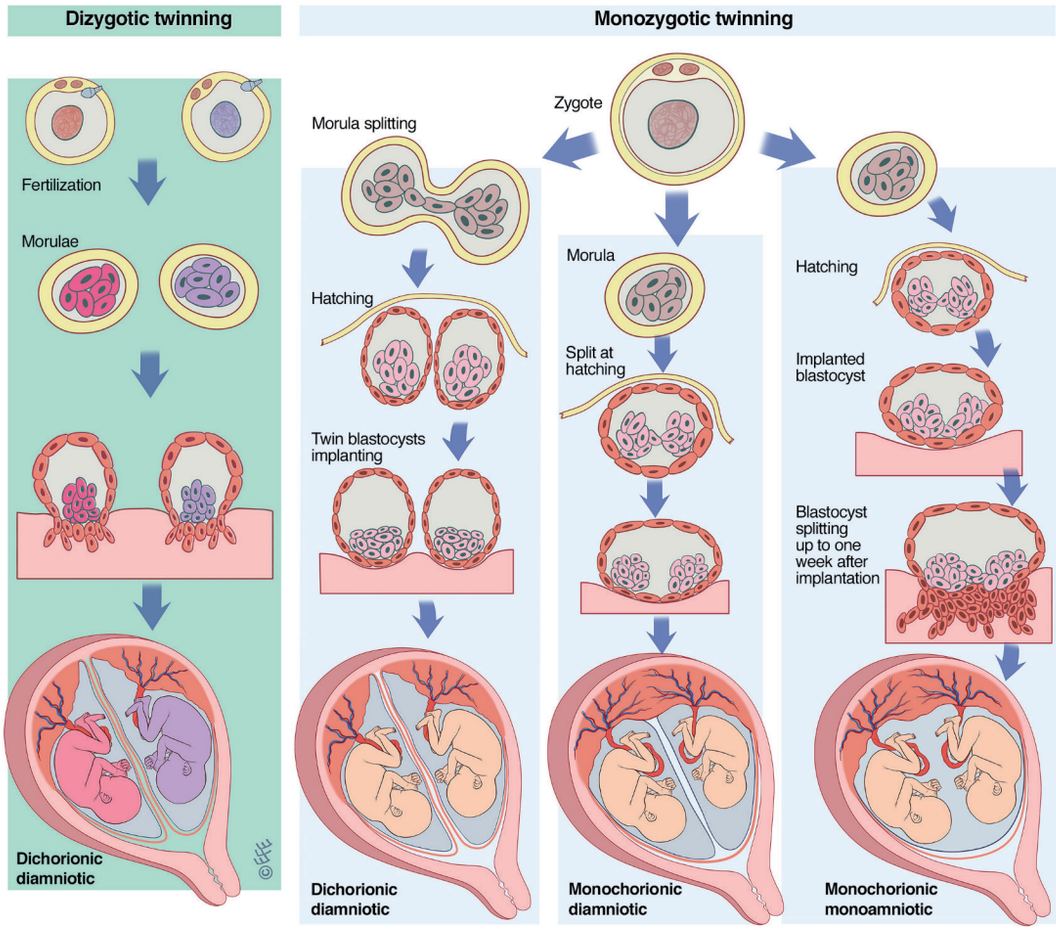
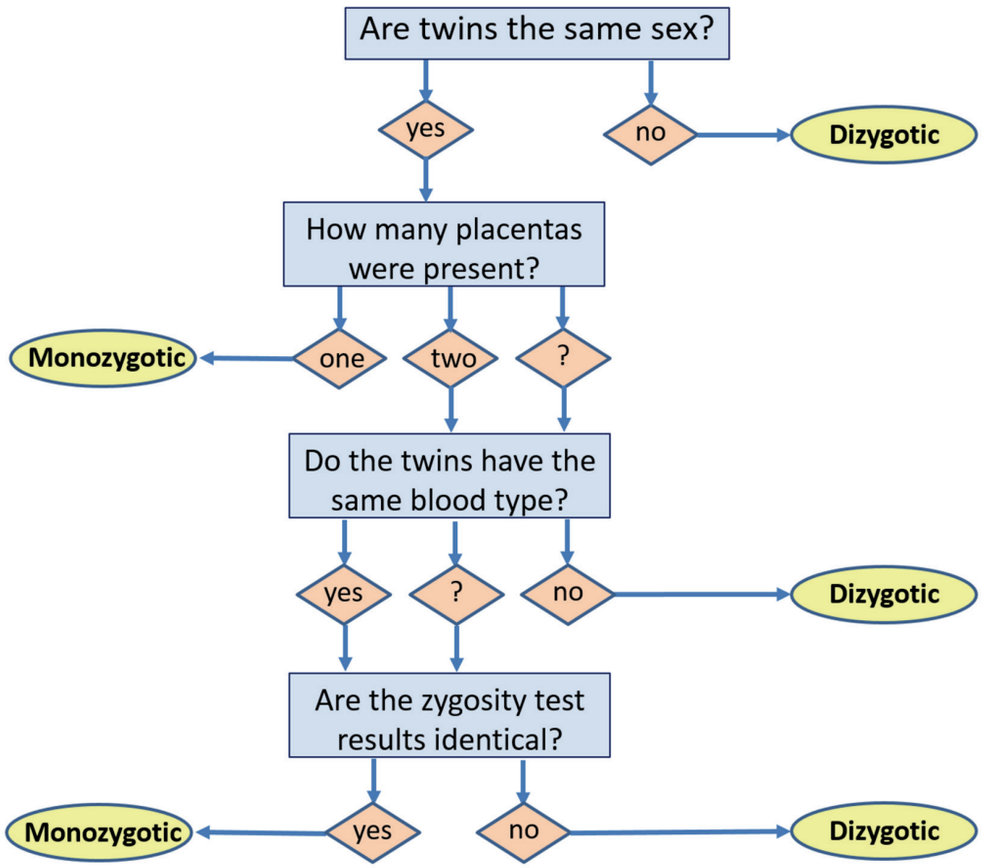


Figure 2. Decision tree for determining zygosity in twins
*Number of placentas should be determined by ultrasound prior to 14 weeks' gestation and confirmed at birth by physical examination of membranes (Kilby, 2017).



form a 'T' shape. Ultrasounds taken later in gestation are less reliable due to the increased crowding of twins in the uterus.

Physical examination of the inter-twin membranes at birth should be used to determine chorionicity (See Figure 3). This will provide confirmation of early ultrasound data and determination of chorionicity in twins where there is no early ultrasound information. Again, dichorionic membranes are thick, opaque and can be pulled apart, whereas monochorionic membranes are thin and semi-transparent.

When sex and chorionicity are known, we can assign different-sex twins as DZ and same-sex monochorionic twins as MZ (with rare exceptions in cases of chromosomal abnormalities) (See Figure 2). When blood type is known, we can assign twins with different blood types as DZ. If chorionicity and blood type are unknown then a test is required to accurately determine zygosity. Same-sex dichorionic twin pairs with the same blood type also require a zygosity test to determine their zygosity.

ZYGOSITY TESTING

Zygosity tests usually involve the analysis of 12-16 highly variable genetic markers (DNA 'fingerprints') which are 100% identical in MZ twins and 40-60% identical in DZ twins. All that is usually needed is a cheek swab, saliva or blood sample, which can be collected by a medical professional or by parents or twins, then sent through the post. The test can be performed quickly and easily at any age, including newborns. The cost for a zygosity test is relatively low; in Australia, parents of twins and adult twins who are members of Twins Research Australia currently pay AU$120.

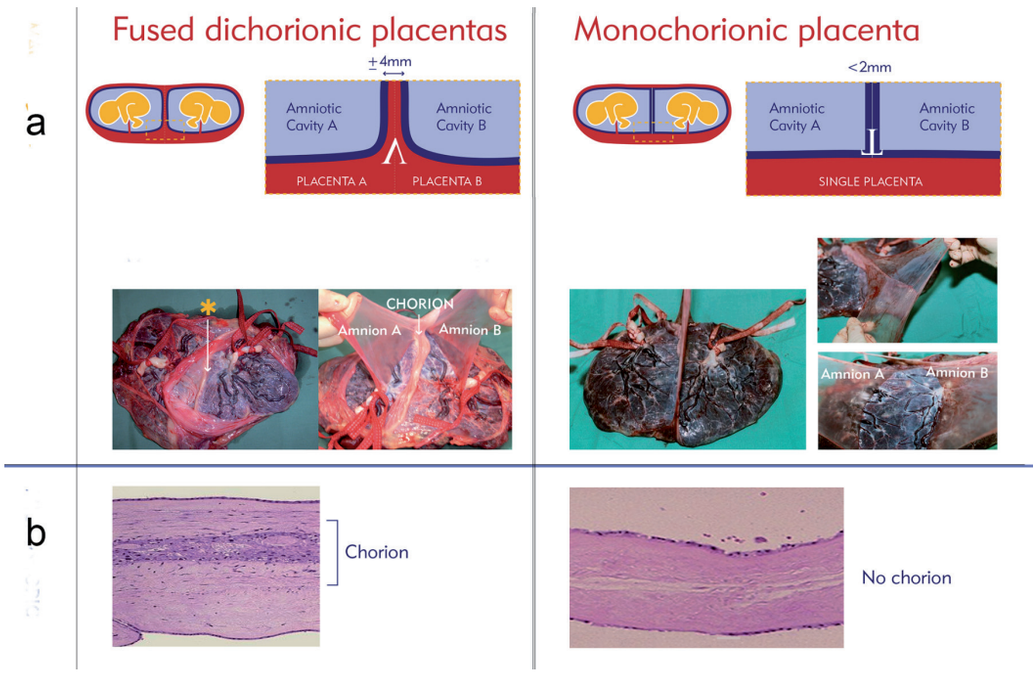
INCORRECT ASSUMPTIONs

The steps in Figure 2 can be used to accurately determine twin zygosity in almost all pairs. Incorrect assumptions lead to misclassification of twins by health care professionals and the twins and their families. The main two incorrect assumptions being firstly that all dichorionic twin pairs are DZ, and secondly that genetically-identical MZ twins must have identical phenotypes.

All DZ twin pairs are dichorionic; however, one third of MZ twin pairs are also dichorionic. The belief that all dichorionic twins are DZ can lead to one third of MZ twins being incorrectly

Figure 3. Determination of chorionicity at birth

Fused dichorionic and monochorionic placentas are compared at the macroscopic (a) and microscopic (b) levels. In fused dichorionic placentas, twins are separated by two amnions (dark blue) and two fused chorions (red). When rubbed between thumb and fingers, the dividing membranes can be separated into two semi-transparent amnions and a thicker layer of fused chorions. The fused chorionic membranes are continuous with the placentas, resulting in a slight ridge on the placental surface (*) corresponding to the lambda sign previously observed on ultrasounds scans (Δ). In monochorionic placentas, twins are separated by two amnions only. When rubbed between thumb and fingers, the dividing membranes are thinner and semi-transparent. The placental surface is flat and the placental junction between the two twins corresponds to the 'T' sign previously observed on ultrasounds scans.



categorised as DZ. The number of chorions can be difficult to determine from ultrasounds. Dichorionic twin pairs may have fused placentas which can be mistaken for a single placenta without careful examination at birth. This can lead to misclassification of DZ pairs as MZ.

MZ twins usually look and behave more similarly than DZ twins due to their greater genetic similarity. However, MZ twins are often not physically and behaviourally identical due to differences in the environments they encounter from conception onwards. Following the splitting of the embryo, with each MZ fetus having the same genes, uterine environmental factors can cause changes to the way the genes work, which can make the twins different. Ninety-nine percent of twins have their own amnion, umbilical cord and (share of the) placenta (See Figure 1), all of which can differ morphologically within pairs.

All this means that many twin pairs begin to experience different environments even when they are in the womb. MZ twins and their parents report that they are acutely aware of any differences in physical appearance, medical conditions and even behaviour between the twins and that these differences lead them to believe the twins could be DZ. Accurate testing and reporting of twin chorionicity and zygosity would conclusively identify whether a set of twins are identical or fraternal.

PHENOTYPE: the person's observable physical characteristics or traits

MORPHOLOGY: study of the specific structural features of organisms

IMPORTANCE OF ZYGOSITY KNOWLEDGE

Accurate knowledge of zygosity is important to twins, parents, clinicians and researchers. There is a range of medical, personal, financial, scientific, legal and ethical reasons supporting greater testing and reporting of twin zygosity (Craig et al., 2016; Keith & Machin, 1997).

MEDICAL

MZ pairs are perfectly compatible organ donors for one another, requiring much less post-transplant immuno-suppression than DZ twins and have better chances of long-term survival.

As nearly all diseases have at least some genetic component, the diagnosis of a disease in one

twin means the co-twin is at increased risk of that disease, more so for MZ than DZ pairs. Genetic sequence data will almost always be the same for MZ co-twins and the implications of this for the co-twin should be considered if testing is undertaken. Knowledge of a genetic disorder manifested in only one of a pair of identical twins is likely to lead to early detection in the second twin, thus leading to a greater chance of successful prevention or treatment for that twin.

There are psychological benefits for parents and twins in knowing whether the twins are MZ or DZ. In the event that one or both twins die before or soon after birth, it is vital that parents and/or the surviving twin have this fundamental information about zygosity as it bears upon immediate bereavement response, the long-term identity of the surviving twin, and future family planning.

PERSONAL

Accurate determination of zygosity is important postnatally for estimation of the likelihood of the mother or close female relatives giving birth to further sets of twins because only DZ twins can run in families (with rare exceptions), mainly through the maternal line.

Knowledge of zygosity also aids in understanding the physical and behavioural differences and similarities between twins. It helps define twins as individuals and their social relationships and can provide peace of mind for twins and their families, avoiding embarrassment when asked about zygosity by family, friends, teachers and strangers. Accurate knowledge is also important because some twins experience significant emotional stress if they discover later in life that their belief about their zygosity was incorrect.

FINANCIAL

Knowledge of a genetic disorder manifested in only one of a pair of identical twins is likely to lead to early detection in the second twin, thus leading to savings in the cost of treatment and management compared to detection at a later stage of disease.

SCIENTIFIC

Knowledge of zygosity is also a prerequisite for twin research and many twins feel they are unable to participate if they are uncertain of their zygosity (Cutler et al., 2015). Inaccurate knowledge of zygosity will affect the results and findings of research.

ETHICAL

In their 'Declaration of Rights' (2014), the International Council for Multiple Birth Organisations (ICOMBO) and the International Society of Twin Studies (ISTS) state that:

> 'Parents have a right to expect accurate recording of placentation, determination of chorionicity and amnionicity via ultrasound, and the diagnosis of zygosity of same sex multiples at birth'.

The Declaration also states that:

'Zygosity should be respected as any other human trait and deserves the same privacy rules'.

In the 'Universal Declaration on the Human Genome and Human Rights' (1997), UNESCO also supports the right to know one's genetic identity/information.

Knowledge of zygosity at birth also avoids erroneous assumptions from the outset. Certainty can be very reassuring, especially for parents (Bamforth & Machin, 2004). For these reasons and those listed above, we argue that not offering twin zygosity testing at birth is unethical.

ARGUMENTS AGAINST TESTING

It has been suggested that it would be inappropriate to undertake zygosity testing on a baby or young child as it removes the individual's ability to decide whether they want to have this information or not (Brown, 2016; Brown, 2015). However, zygosity testing is quite distinct from other forms of genetic testing of children that raise legitimate ethical concerns. Most notably, it is not a test for a gene mutation or predisposition to disease. It therefore cannot be compared with testing for predisposition to a genetic disease, particularly for a late-onset condition such as Huntington's disease, for which it is always preferable to let the individual decide for themselves whether to undertake testing.

Importantly, we are not recommending mandatory zygosity testing for twins. In the case of infants and young children, some parents may not wish to know.

IMPACT OF ZYGOSITY KNOWLEDGE ON TWINS

In one study, twins were asked why it was important for them to know their zygosity (Cutler et al., 2015). Some of the responses are below.

'For health reasons, I feel it was necessary to know so if one was to have a disease etc. we would pay closer attention to the other twin.' (Mother of MZ twins aged 2, previously unsure of their zygosity)

'[It meant] the world! I felt close to my sister and was always really curious to know if we were actually identical or not, so finally finding out was like settling a piece of unknown family history. It was fabulous.' (MZ twin aged 25, previously believed she was a DZ twin)

'Assurance of total compatibility and similarity for medical reasons.' (MZ twin aged 40, previously guessed he was MZ)

THE IMPORTANCE OF TWINS IN RESEARCH

The study of twins helps disentangle genetic from environmental influences on human traits and conditions as identical twins share all of their genetic information while non-identical twins share around half. In relation to health conditions that are affected to a high

degree by genetics, identical twins will be much more alike than non-identical twins.

Conversely, if a health condition is largely affected by the family environment (for example living in dire poverty), then both MZ and DZ twins will be similarly affected.

Research involving twins has been conducted for decades (Hur & Craig, 2013). The research has proved invaluable in helping to separate the effects of genes and environment on variation in human characteristics, behaviours, and susceptibility to diseases. We argue that increasing the accuracy of zygosity reporting will increase the accuracy and validity of twin research.

RECOMMENDATIONS

Recommendation 1
Increase education for professionals including obstetricians, neonatologists, midwives, maternal child health nurses, paediatricians, ultrasonographers and perinatal educators, about twin zygosity, its testing and its implications.

Recommendation 2
All parents of newborn twins should be provided with a written obstetric report of their twins' chorionicity and its implications for zygosity. If their twins are the same sex and dichorionic and they wish to know the zygosity, they should be provided with enough information to make an informed decision about whether to undergo zygosity testing.

Recommendation 3
Universal zygosity testing of same-sex twins should be encouraged as early in life as possible as standard practice. Parents of same-sex twins and their families should always be advised that if they wish to know their twins' zygosity, the only way to be certain, except in situations where twins have different blood groups, is to have a zygosity test.

Recommendation 4
From birth, all children should be recorded as a singleton or a twin in medical records. This way, if one twin sees a medical professional subsequently, issues related to risk to the co-twin can be raised. Such information is also essential for data linkage and twin research.

Recommendation 5
Parents of twins should be asked by medical professionals if they know whether their twins are identical or fraternal and how they know. Those who are unsure, or who have made an incorrect assumption, should be encouraged to have a zygosity test. They should receive accessible and accurate information about the tests and the costs involved.

Recommendation 6
Twins and their families should receive education about zygosity and its implications. Families of twins should be provided with details of local and national twin registries and multiple birth organisations for reasons of peer support and contribution to research.

Recommendation 7
With the help of government and genetic testing companies, the burden of the cost of zygosity testing should be reduced. Currently in Australia, 1.5% of all births are twins and the most commonly accessed zygosity test costs AUD$120. Therefore, the annual, national cost of zygosity testing for all same-sex dichorionic twins would be around AUD$230,000 or AUD$345,000 for all same-sex twins irrespective of chorionicity. We submit that this is a modest cost given the potential significance of the information gained.

Recommendation 8
A study of the frequency of misattributed zygosity in untested twins should be undertaken to determine the scale of the problem.

Recommendation 9
A study of the health benefits and cost effectiveness of zygosity testing should be undertaken to provide evidence for funding of zygosity testing through the public health system.

Recommendation 10
A higher rate of family payments should be provided to families with twins in recognition of the elevated cost of raising twins (five times more than singletons in the USA: Lemos et al., 2013)). This approach would be consistent, in Australia, with the multiple birth allowance for triplets and quads and would give families more resources which they could use for zygosity testing if they chose.

ACKNOWLEDGEMENTS

The authors acknowledge grants from the Centre of Research Excellence (1079102) of the National Health and Medical Research Council of Australia. JMC is supported by grants from the Australian National Health and Medical Research Council (grant numbers 1011070 and 1083779), the Financial Markets Foundation For Children (grant number 032-2007), and the Murdoch Children's Research Institute, which is funded by the Victorian Government's Operational Infrastructure Support Program.

REFERENCES

Bajoria, R., Kingdom, J. (1997) The case for routine determination of chorionicity and zygosity in multiple pregnancy. Prenatal Diagnosis, 17:1207-25.

Bamforth, F., Machin, G. (2004) Why zygosity of multiple births is not always obvious: An examination of zygosity testing requests from twins or their parents. Twin Research: The Official Journal of the International Society for Twin Studies, 7:406-11.

Brown, R. (2015) Zygosity testing should be encouraged for all same-sex twins: AGAINST:

Benefit of this knowledge should be weighed against potential pitfalls. British Journal of Obstetrics and Gynaecology, 122:1641.

Brown, R. (2016) Author's reply re: Zygosity testing should be encouraged for all same-sex twins. AGAINST: The benefit of this knowledge should be weighed against the potential pitfalls. British Journal of Obstetrics and Gynaecology, 123:1561.

Craig, J.M., All, A. (2016) Re: Zygosity testing should be encouraged for all same-sex twins. AGAINST: The benefit of this knowledge should be weighed against the potential pitfalls. British Journal of Obstetrics and Gynaecology, 123:1560-1.

Craig, J.M., Segal, N.L., Umstad, M.P., Cutler, T.L., Keogh, L.A. et al. (2015) Zygosity testing should be encouraged for all same-sex twins: FOR: A genetic test is essential to determine zygosity. British Journal of Obstetrics and Gynaecology, 122:1641.

Cutler, T.L., Murphy, K., Hopper, J.L., Keogh, L.A., Dai, Y. et al. (2015) Why accurate knowledge of zygosity is important to twins. Twin Research and Human Genetics, 18:298-305.

Derom, R., Vlietinck, R.F., Derom, C., Keith, L.G., Van Den Berghe, H. (1991) Zygosity determination at birth: A plea to the obstetrician. Journal of Perinatal Medicine, 19, Suppl 1: 234-40.

Hall, J.G. (2003) Twinning. Lancet, 362:735-43.

Hur, Y.M., Craig, J.M. (2013) Twin registries worldwide: An important resource for scientific research. Twin Research and Human Genetics, 16:1-12.

ICOMBO (International Council of Multiple Birth Organisations) and ISTS (International Society for Twin Studies) (2014) Declaration of Rights and Statement of Needs of Twins and Higher Order Multiples. Available at: (http://icombo.org/wp-content/uploads/2010/11/Declaration-of-Rights-2014.pdf) <accessed 29 Dec, 2019>

Keith, L., Machin, G. (1997) Zygosity testing: Current status and evolving issues. Journal of Reproductive Medicine, 42:699-707.

Kilby, M.D. (2017) Management of Monochorionic Twin Pregnancy: Green-top Guideline No. 51. British Journal of Obstetrics and Gynaecology, 124,:e1-e45.

Lemos, E.V., Zhang, D., Van Voorhis, B.J., Hu, X.H. (2013) Healthcare expenses associated with multiple vs singleton pregnancies in the United States. American Journal of Obstetrics and Gynecology, 209(6):e1-586 e11.

Machin, G.A. (2004) Why is it important to diagnose chorionicity and how do we do it? Best Practice and Research: Clinical Obstetrics and Gynaecology, 18:515-30.

Machin, G. (2009) Non-identical monozygotic twins, intermediate twin types, zygosity testing, and the non-random nature of monozygotic twinning: A review. American Journal of Medical Genetics, Part C Seminars in Medical Genetics, 151C(2):110-27.

Maruotti, G.M., Saccone, G., Morlando, M., Martinelli, P. (2016) First-trimester ultrasound determination of chorionicity in twin gestations using the lambda sign: A systematic review and meta-analysis. European Journal of Obstetrics and Gynecology and Reproductive Biology, 202:66-70.

McNamara, H.C., Kane, S.C., Craig, J.M., Short, R.V., Umstad, M.P. (2016) A review of the mechanisms and evidence for typical and atypical twinning. American Journal of Obstetrics and Gynecology, 214:172-91.

Segal, N.L. (1984) Zygosity testing: Laboratory and the investigator's judgment. Acta Geneticae Medicae et Gemellologiae (Roma), 33:515-21.

UNESCO (United Nations Educational, Scientific and Cultural Organization) (1997) Universal Declaration on the Human Genome and Human Rights. Available at: www.ohchr.org/EN/ProfessionalInterest/Pages/HumanGenomeAndHumanRights.aspx <accessed 29 Dec, 2019>

Van Jaarsveld, C.H., Llewellyn, C.H., Fildes, A., Fisher, A., Wardle, J. (2012) Are my twins identical? Parents may be misinformed by prenatal scan observations. British Journal of Obstetrics and Gynaecology, 119:517-8.