Review

Gestational Surrogacy and the Role of Routine Embryo Screening: Current Challenges and Future Directions for Preimplantation Genetic Testing

E. Scott Sills*[1,2], Robert E. Anderson[3], Mary McCaffrey[4], Xiang Li[5], Nabil Arrach[6,7], and Samuel H. Wood[6,7]

Preimplantation genetic screening (PGS) is a component of IVF entailing selection of an embryo for transfer on the basis of chromosomal normalcy. If PGS were integrated with single embryo transfer (SET) in a surrogacy setting, this approach could improve pregnancy rates, minimize miscarriage risk, and limit multiple gestations. Even without PGS, pregnancy rates for IVF surrogacy cases are generally satisfactory, especially when treatment utilizes embryos derived from young oocytes and transferred to a healthy surrogate. However, there could be a more general role for PGS in surrogacy, since background aneuploidy in embryos remains a major factor driving implantation failure and miscarriage for all infertility patients. At present, the proportion of IVF cases involving GS is limited, while the number of IVF patients requesting PGS appears to be increasing. In this report, the relevance of PGS for surrogacy in the rapidly changing field of assisted fertility medicine is discussed.

Birth Defects Research (Part C) 108:98–102, 2016.

Key words: IVF; gestational surrogacy; preimplantation genetic screening; single embryo transfer

Introduction

Gestational surrogacy (GS) is a medical solution to involuntary childlessness in settings where the patient's uterus is abnormal, absent, or cannot safely be used to establish a pregnancy for medical reasons. Patients may also seek GS in any medical context where pregnancy is contraindicated, or where two males seek to become parents through oocyte donation and IVF. Advances in IVF have brought surrogacy to a high level of sophistication, attracting notice (sometimes unwelcome) from voices in legislative, ethical, religious, and popular culture domains. Occasional controversy notwithstanding, the pregnancy and delivery rates among IVF patients undergoing GS are generally quite good (Brinsden, 2003; Raziel et al., 2012).

The process of GS always operates against a background of IVF, and entails a woman agreeing to become pregnant (via embryo transfer) and then carrying the pregnancy to term with the intent to relinquish custody of that child upon its birth to the patient/s with whom she has made the contract (Sheean et al., 1989). This agreement has the practical effect of causing a purposeful conception followed by planned, voluntary surrender of the offspring by the birth mother, at or shortly after birth (van den Akker, 2007). While the surrogate may have a purely altruistic reason for agreeing to carry a child for someone else, this service (in California) is usually done in exchange for monetary compensation to the surrogate mother. Recent research has challenged the cultural assumption that "normal" women do not voluntarily become pregnant with the premeditated intent to surrender the child for money (Teman, 2003). Regarding the level of support for surrogacy, considerable variation exists across various groups with some of this perception appearing to be influenced by local media portrayals of this medical treatment (Appleton, 2001).

As with all assisted reproduction technologies, the ultimate aim of GS is a healthy, singleton delivery. And as with all IVF cases, advanced maternal age is the most critical determinant for the success rate in GS. The embryo implantation failure rate due to aneuploidy likely exceeds 50% when the female is older than 38 years of age (Franasiak et al., 2014). From maternal, neonatal, social, and economic perspectives, the single embryo transfer (SET) is unquestionably the overall best strategy to prevent multiple

---

The authors declare no conflicts of interest.

[1]Reproductive Research Section, Center for Advanced Genetics, Carlsbad, California
[2]Faculty of Applied Biotechnology, School of Life Sciences, University of Westminster, London, United Kingdom
[3]Southern California Center for Reproductive Medicine, Newport Beach, California
[4]The Scotia Clinic, Tralee, County Kerry, Ireland
[5]Division of Analytics & Quantitative Research, Rosenblatt Securities Inc, New York, New York
[6]Reproductive Sciences Center, San Diego, California
[7]Progenesis, La Jolla, California

*Correspondence to: E. Scott Sills; Center for Advanced Genetics, 3144 El Camino Real, Suite 106, Carlsbad, California 92008. E-mail: drsills@CAGivf.com

Published online 23 November 2015 in Wiley Online Library (wileyonlinelibrary.com). Doi: 10.1002/bdrc.21112

gestation and preterm birth (Sills, 2013). Aware of the substantial impact of genetic pathology in embryos, providing an effective option for SET to patients undergoing GS will be contingent on improved methods of embryo selection (Schoolcraft and Katz-Jaffe, 2013; Wu et al., 2014).

Routine Preimplantation Genetic Screening (PGS) in Gestational Surrogacy

As for any patient who undergoes IVF, pregnancies from GS also should be ideally achieved with the transfer of a single embryo (SET). At present, SET is far from routine in California, since most IVF units allow (if not encourage) multiple embryos to be transferred at the same time. This approach helps IVF clinics keep overall pregnancy success rates higher than if standard SET were offered, because an individual embryo selected for transfer on the basis of morphologic criteria alone will often fail to implant.

Of note, substantial improvements in comprehensive chromosome screening now permit better informed embryo selection, by providing the necessary tools to identify one euploid embryo for transfer (Wu et al., 2014). One recent randomized controlled trial assessed the benefits of PGS on implantation and pregnancy rates in a standard IVF context (Scott et al., 2013). Women younger than age 42 years with normal ovarian function (and no more than one prior IVF failure) were eligible to enroll. In addition, study patients needed at least two good-morphology blastocysts for randomization into either the PGS or morphologic (conventional) selection groups. Among these patients, the implantation rate was substantially higher in the PGS group (79.8 vs. 63.2%), and the PGS group also had higher clinical pregnancy rates compared with controls (93.1 vs. 80.7%) (Scott et al., 2013). Because embryonic genetic factors also contribute to some IVF failures for GS patients, such observations reported with PGS benefits for standard IVF should generalize to GS patients as well.

The latest technique used to evaluate embryos before transfer is known as "next-generation sequencing" (NGS). In 2011, an NGS study demonstrated the clinical and analytic validity of exome sequencing targeting a preselected region known to be associated with severe childhood autosomal recessive diseases, like Tay–Sachs disease and cystic fibrosis (Bell et al., 2011). Yin et al. (2013) introduced a method of massively parallel sequencing for aneuploidy of blastocysts, reporting a 68.4% euploidy rate. These observations were confirmed by SNP array with high (97.4%) consistency. Tan et al. (2014) published results with NGS-based pre-implantation genetic diagnosis/screening, concluding that the approach provides an accurate method to detect imbalanced segmental rearrangements in the embryonic genome. Moreover, Treff et al. (2013) used NGS to identify single gene mutations in embryos, and validated this with 100% diagnostically equivalency to qPCR. Such results indicate that preimplantation embryo evaluation using NGS can provide highly accurate sequencing information by increasing the read depth (Wu et al., 2014).

While the ovarian age/source of eggs is the principal factor influencing pregnancy rate in GS (as it is with standard two-party IVF), even when the oocyte source in GS is very young, the incidence of embryo aneuploidy can be surprisingly high. To estimate the magnitude of embryonic aneuploidy challenges in GS, a closely related clinical context may provide helpful data. For example, when oocytes are obtained from young, healthy, anonymous egg donors, what is the observed chromosomal error rate in this population? When a few chromosomes were sampled in embryos obtained from donor-egg IVF cycles, the aneuploidy rate was not low (Munné et al., 2006). In 2014, this investigation of embryo ploidy was extended to include all 23 pairs of chromosomes in a California donor egg IVF population (Sills et al., 2014). From more than 300 embryos evaluated, euploidy was present in only 133 (46.8%). Although the oocyte donors were young in this study population (mean age = 24 years), embryonic genetic error attributed to the egg source accounted for 88.1% of all observed aneuploidies (Sills et al., 2014). This finding suggests a remarkably high background aneuploidy rate in human embryos, even when these embryos originate from eggs retrieved from young women with no known infertility diagnosis. This observation has particular relevance for GS patients, whose surrogate may not conceive after embryo transfer, simply due to random imbalances in embryo genetics. Although prospective clinical trials will be required to quantify the beneficial effect, it is plausible that incorporation of PGS with GS would result in reduced miscarriage rates and improved delivery outcomes for these IVF patients, as well.

PGS and The Special Case of MRKH

One diagnosis exceptionally well suited to GS is Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome (Ben-Rafael et al., 1998; Raziel et al., 2012). MRKH is characterized by normal ovarian development, secondary female sexual characteristics, and by a normal 46,XX female karyotype, but with congenital aplasia of the uterus and proximal two-thirds of the vagina (Morcel et al., 2008). MRKH has an incidence of approximately 1:4500 phenotypic females. The syndrome is divided into two types, Type I (simple) and Type II (complex), depending on the presence of various anomalies outside the reproductive system. For example, patients with MRKH Type II may have renal dysplasia and cervical somite anomalies (MURCS) with unilateral renal agenesis, renal ectopia or horseshoe kidney, vertebral anomalies (Klippel–Feil syndrome) including scoliosis (Fisher et al., 2000), anomalies of auditory system, cardiac defects, and syndactyly/polydactyly (Griffin et al., 1976). Of note, MRKH Type I (isolated type) occurs with much lower frequency than the complex type (MRKH Type II); neither form appears to have

any racial predisposition. Although the disorder is congenital, it is not routinely diagnosed until adolescence when primary amenorrhea is revealed.

During patient counseling before GS, an important topic which must be addressed concerns the potential for MRKH to be transmitted to the patient's own genetic offspring. Unfortunately, there is little published longitudinal research on children conceived by MRKH mothers who used their own oocytes with IVF and GS. While experts have given a generally reassuring view of the treatment (Batzer et al., 1992), data from long-term surveillance is still needed for a more complete understanding of intergenerational MRKH expression. Until the arrival of GS with IVF, women with MRKH had no chance to create a genetically connected family. The worldwide number of MRKH offspring now is unknown, and the uncertainty of the etiology of MRKH has helped drive a keen interest in genetic mapping of the disorder among affected patients.

PGS AND MRKH: UNANSWERED QUESTIONS

Although MRKH may be regarded as the classic anatomical problem where GS supplies a medical solution, the uncertainty regarding the genetic basis of MRKH has resulted in an inability to utilize preimplantation embryo testing for these patients thus far. While MRKH was once classified as a sporadic anomaly, more recent work has suggested an autosomal dominant inheritance pattern with incomplete penetrance and variable expressivity (Morcel et al., 2008). In the search for putative genetic causes for this syndrome, attention is understandably focused on mutations known to manifest their effect during the early phase of embryo development. Wilms' tumor 1 (WT1), paired box 2 (PAX2), certain homeotic genes (i.e., HOXA7–HOXA13), and pre-B-cell leukemia homeobox 1 (PBX1) have each been implicated in the etiology of MRKH (Acién et al., 2004).

WNT4 has been strongly associated with MRKH, as this gene specifically directs female genital development during embryogenesis (Biason-Lauber et al., 2004; Drummond et al., 2008; Sultan et al., 2009). In the murine model, three members of the WNT gene family (WNT4, WNT5a, and WNT7a) are expressed at high levels in the developing female genital tract (Miller et al., 1998a). Homozygotic inactivation of these genes results in a range of severe Müllerian ducts defects, including a malformation similar to that seen with in utero DES exposure (Miller et al., 1998b) to total failure of Müllerian duct formation, and even lethal developmental defects (Vainio et al., 1999). This WNT family is comprised of structurally correlated genes, whose proteins are involved in cellular differentiation as well as other crucial developmental processes. Indeed, WTNT4 plays a determinative role both in the induction of female gonads and in blocking testicular formation.

It has been proposed (Jordan et al., 2001) that WNT4 is the upstream signal triggering a cascade of events culminating in sexual determination, mediated by local growth factors. A knock-out of this gene in offspring results in secondary male phenotype, even if the genotype is female. Such observations have placed WNT4 forward in the group of genes thought to be responsible for proper organization of the female sexual organs. Unfortunately, detailed screening for mutations of some genes in MRKH patients initially failed to identify any consistent alteration which could be regarded as causative (Bernardini et al., 2009). Subsequent work (Ledig et al., 2011) found three regions (1q21.1, 17q12, and 22q11.21) of particular interest, suggesting that deletions and/or missense mutations in LHX1 and HNF1B are also strong gene candidates in MRKH.

With respect to the role of HOX genes in MRKH, it is notable that deletion of the entire HOXA cluster does not result in disruption of the entire reproductive tract, contrary to what happens with more limited, single monoallelic HOXA13 mutations (Devriendt et al., 1999). Thus, either monoallelic dominant mutations within HOXA9/10/11 might be causing the MRKH phenotype, or perhaps other disruptions of HOX are altering its transcription (Innis, 2002; Guerrier et al., 2006).

Although these are important and encouraging early results, more work remains to be done. If such findings can be validated by other investigators, then not only might targeted gene therapy for MRKH patients become a reality (Connell et al., 2013), but it also should be possible to develop a "MRKH panel" for preimplantation genetic diagnosis and apply this assessment to embryos before transfer in IVF and GS. NGS will be especially well-suited for this application as it can be easily configured to detect many mutations with high accuracy.

Conclusion

IVF with GS remains a highly effective treatment for many patients with an absent or impaired uterus. There is certainly a role for PGS whenever a discrete genetic diagnosis exists in the commissioning parent, although there may also be a role for PGS in gestational surrogacy even when the "genetic mother" is healthy and relatively young (i.e., age $<$ 35 years). In other words, while a youthful oocyte source in GS is welcome and may be associated with a partial reduction of the embryo aneuploidy problem, the identification and exclusion of all embryos with genetic errors is not presently possible. As the appreciation of generalized embryo aneuploidy grows among patients considering GS, a further expanded use of PGS into the domain of gestational surrogacy seems likely.

References

Acién P, Acién M, Sánchez-Ferrer M. 2004. Complex malformations of the female genital tract: new types and revision of classification. Hum Reprod 19:2377–2384.

Appleton T. 2001. Surrogacy. Curr Obstet Gynecol II 256–257.

Batzer FR, Corson SL, Gocial B, et al. 1992. Genetic offspring in patients with vaginal agenesis: Specific medical and legal issues. Am J Obstet Gynecol 167:1288–1292.

Bell CJ, Dinwiddie DL, Miller NA, et al. 2011. Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med 3:65ra64.

Ben-Rafael Z, Bar-Hava I, Levy T, et al. 1998. Simplifying ovulation induction for surrogacy in women with Mayer-Rokitansky-Kuster-Hauser syndrome. Hum Reprod 13:1470–1471.

Bernardini L, Gimelli S, Gervasini C, et al. 2009. Recurrent microdeletion at 17q12 as a cause of Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome: Two case reports. Orphanet J Rare Dis 4:(1, article 25).

Biason-Lauber A, Konrad D, Navratil F, et al. 2004. A WNT4 mutation associated with Müllerian-Duct regression and virilization in a 46,XX woman. N Engl J Med 351:792–798.

Brinsden PR. 2003. Gestational surrogacy. Hum Reprod Update 9:483–491.

Connell M, Owen C, Segars J. 2013. Genetic syndromes and genes involved in the development of the female reproductive tract: A possible role for gene therapy. J Genet Syndr Gene Ther 4:127.

Devriendt K, Jaeken J, Matthijs G, et al. 1999. Haploinsufficiency of the HOXA gene cluster, in a patient with hand-foot-genital syndrome, velopharyngeal insufficiency, and persistent patent Ductus botalli. Am J Hum Genet 65:249–251.

Drummond JB, Reis FM, Boson WL, et al. 2008. Molecular analysis of the WNT4 gene in 6 patients with Mayer-Rokitansky-Küster-Hauser syndrome. Fertil Steril 90:857–859.

Fisher K, Esham RH, Thorneycroft I. 2000. Scoliosis associated with typical Mayer-Rokitansky-Küster-Hauser syndrome. South Med J 93:243–246.

Franasiak JM, Forman EJ, Hong KH, et al. 2014. The nature of aneuploidy with increasing age of the female partner: A review of 15, 169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril 101:656–663.

Griffin JE, Edwards C, Madden JD. 1976. Congenital absence of the vagina. The Mayer-Rokitansky-Küster-Hauser syndrome. Ann Int Med 85:224–236.

Guerrier D, Mouchel T, Pasquier L, et al. 2006. The Mayer-Rokitansky-Küster-Hauser syndrome (congenital absence of uterus and vagina)–phenotypic manifestations and genetic approaches. J Neg Results Biomed 5:1.

Innis JW. 2002. Priming the search for HOX mutations. Teratology 65:47–49.

Jordan BK, Mohammed M, Ching ST, et al. 2001. Up-regulation of WNT-4 signaling and dosage-sensitive sex reversal in humans. Am J Hum Genet 68:1102–1109.

Ledig S, Schippert C, Strick R, et al. 2011. Recurrent aberrations identified by array-CGH in patients with Mayer-Rokitansky-Küster-Hauser syndrome. Fertil Steril 95:1589–1594.

Miller C, Degenhardt K, Sassoon DA. 1998a. Fetal exposure to DES results in de-regulation of Wnt7a during uterine morphogenesis. Nat Genet 20:228–230.

Miller C, Pavlova A, Sassoon DA. 1998b. Differential expression patterns of Wnt genes in the murine female reproductive tract during development and the estrous cycle. Mech Dev 76: 91–99.

Morcel K, Guerrier D, Watrin T, et al. 2008. The Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome: Clinical description and genetics. J Gynecol Obstet Biol Reprod 37:539–546.

Munné S, Ary J, Zouves C, et al. 2006. Wide range of chromosome abnormalities in the embryos of young egg donors. Reprod Biomed Online 12:340–346.

Raziel A, Friedler S, Gidoni Y, et al. 2012. Surrogate in vitro fertilization outcome in typical and atypical forms of Mayer-Rokitansky-Kuster-Hauser syndrome. Hum Reprod 27:126–130.

Schoolcraft WB, Katz-Jaffe MG. 2013. Comprehensive chromosome screening of trophectoderm with vitrification facilitates elective single-embryo transfer for infertile women with advanced maternal age. Fertil Steril 100:615–619.

Scott RTJ, Upham KM, Forman EJ, et al. 2013. Blastocyst Biopsy With Comprehensive Chromosome Screening and Fresh Embryo Transfer Significantly Increases In Vitro Fertilization Implantation and Delivery Rates: A Randomized Controlled Trial. Fertil Steril 100:697–703.

Sheean LA, Goldfarb JM, Kiwi R, Utian WH. 1989. In vitro fertilization (IVF)-surrogacy: Application of IVF to women without functional uteri. J In Vitro Fert Embryo Transf 6: 134–7.

Sills ES. 2013. An evidence-based policy for the provision of subsidised fertility treatment in California: Integration of array comparative genomic hybridisation with IVF and mandatory single embryo transfer to lower multiple gestation and preterm birth rates [PhD thesis, Univ Westminster-London. British Library EthOS uk.bl.ethos.576982].

Sills ES, Li X, Frederick JL, Khoury CD, et al. 2014. Determining parental origin of embryo aneuploidy: Analysis of genetic error observed in 305 embryos derived from anonymous donor oocyte IVF cycles. Mol Cytogenet 7:68.

Sultan C, Biason-Lauber A, Philibert P. 2009. Mayer-Rokitansky-Küster-Hauser syndrome: Recent clinical and genetic findings. Gynecol Endocrinol 25:8–11.

Tan Y, Yin X, Zhang S, et al. 2014. Clinical outcome of preimplantation genetic diagnosis and screening using next generation sequencing. Gigascience 3:30.

Teman E. 2003. The medicalization of "nature" in the "artificial body": Surrogate motherhood in Israel. Med Anthropol Q 17:78–98.

Treff NR, Fedick A, Tao X, et al. 2013. Evaluation of targeted next-generation sequencing-based preimplantation genetic diagnosis of monogenic disease. Fertil Steril 99:1377–1384.

Vainio S, Heikkila M, Kispert A, et al. 1999. Female development in mammals is regulated by Wnt-4 signalling. Nature 397:405–409.

van den Akker OB. 2007. Psychosocial aspects of surrogate motherhood. Hum Reprod Update 13:53–62.

Wu MY, Chao KH, Chen CD, et al. 2014. Current status of comprehensive chromosome screening for elective single-embryo transfer. Obstet Gynecol Int 1:581783.

Yin X, Tan K, Vajta G, et al. 2013. Massively parallel sequencing for chromosomal abnormality testing in trophectoderm cells of human blastocysts. Biol Reprod 88:69.