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ORIGINAL ARTICLE

PREIMPLANTATION GENETIC TESTING WITHIN THE PUBLIC HEALTHCARE SYSTEM IN SLOVENIA

Volk M[1], Writzl K[1], Veble A[1], Jaklič H[1], Teran N[1], Prosenc B[1], Štimpfel M[2], Virant Klun I[3], Vrtačnik Bokal E[2], Ban Frangež H[2], Peterlin B[1,*]

[*]**Corresponding Author:** Prof. Borut Peterlin, MD, PhD, Clinical institute of genomic medicine UMC Ljubljana, Šlajmerjeva 004, 1000 Ljubljana, Slovenia, Telephone: +3861 5226103, Fax: +3861 5401137, borut.peterlin@kclj.si

ABSTRACT

Preimplantation genetic testing (PGT) is the earliest form of prenatal diagnosis that has become an established procedure for couples at risk of passing a severe genetic disease to their offspring. At UMC Ljubljana, we conducted a retrospective register-based study to present 15 years of PGT service within the public healthcare system in Slovenia. We collected the data of the PGT cycles from 2004 to 2019 and compared clinical outcomes for chromosomal and monogenic diseases using different embryo biopsy and testing approaches. In addition, we assessed the extent to which PGT has become the preferred option compared to classic prenatal diagnostics. We treated 211 couples, 110 with single gene disorder, 88 with structural chromosome rearrangement and 13 for numerical chromosome aberration. There were 375 PGT cycles with oocyte retrieval, while embryo transfer was possible in 263 cases resulting in 78 deliveries and 84 children. Altogether, the clinical pregnancy rate per embryo transfer was 31% in 2004-2016 (blastomere biopsy) and 43% in 2017-19 (blastocyst biopsy), respectively. We assessed that approximately a third of couples would opt for PGT, while the rest preferred natural conception with prenatal diagnosis. Our results show that providing a PGT service within the public healthcare system has become a considerable option in pregnancy planning for couples at risk of transmitting a severe genetic disease to their offspring. In Slovenia, approximately a third of couples would opt for PGT. Although the number of cycles is small, our clinical results are comparable to larger centres.

Keywords: chromosome aberration, embryo biopsy, in vitro fertilization, monogenic disease, preimplantation genetic testing.

INTRODUCTION

Preimplantation genetic testing (PGT) is an established procedure for couples at risk of transmitting a genetic disease to their offspring. PGT involves in vitro fertilization using ICSI and genetic analysis of the embryo prior to transfer and implantation. Such practice allows the selection of an unaffected embryo for the specific pathogenic variant tested, thus avoiding the termination of pregnancy following classical prenatal diagnostic testing. The first PGT procedure was performed in 1990 for sex selection of X-linked disorder in the United Kingdom (1, 2). With advances in assisted reproductive technology (ART) and molecular genetic methods, PGT has become an essential reproductive option as it significantly reduces the risk of affected offspring. Another motive for PGT is the reduced psychological burden and uncertainty of future parents.

PGT can be performed for any severe monogenic disease (PGT-M) or chromosome rearrangement (PGT-SR). In addition, preimplantation aneuploidy screening (PGT-A, formerly preimplantation genetic screening - PGS) is applied worldwide in a subgroup of infertile patients with normal karyotypes undergoing in vitro fertilization (3). Although the technical procedures for PGT-M, PGT-SR and PGT-A are similar, the indications differ. In Slove-

[1] Clinical Institute of Genomic Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia.

[2] Department of Human Reproduction, Division of Gynaecology and Obstetrics, University Medical Centre Ljubljana, Ljubljana, Slovenia.

[3] Clinical Research Centre, University Medical Centre Ljubljana, Ljubljana, Slovenia.

nia, prospective parents at risk of transmitting structural chromosome rearrangement or monogenic disease to their offspring may opt for PGT-SR or PGT-M, while PGT-A for infertile couples is not routinely practiced. However, PGT-A may be performed if a parent is a carrier of numerical chromosome aberration.

While modern PGT methodologies, performance, and outcomes of PGT services are similar between individual centres, the practices of how and to whom to offer PGT vary between countries depending on different jurisdictions and policy approaches (4). There is also limited data on what proportion of patients would opt for PGT as a first choice for testing instead of natural conception with classical prenatal diagnostics.

We conducted a retrospective register-based study to present 15 years of development and provision of PGT within the public healthcare system in Slovenia.

MATERIALS AND METHODS

Subjects

Two hundred and eleven (211) couples with a known genetic predisposition, 110 with monogenic disorder, 88 with chromosome structural rearrangement, and 13 with mosaic sex or numeric chromosome abnormality, were eligible for the PGT procedure. Ovarian stimulation and oocyte retrieval were performed according to standard protocol (5). All couples signed informed consent prior to the PGT procedure. Clinical operations have been conducted following the principles expressed in the Helsinki declaration.

Methods

Preimplantation genetic testing was implemented in 2004. Prior to enrolment, the couples underwent genetic counselling. Genetic counselling is organized stepwise to provide all the relevant education and information associated with the procedure. Couples attending PGT cycles are informed about the benefits, limitations, and risks of the PGT procedure and the expected delivery rate per embryo transfer. The multidisciplinary approach manages coordination between hormone stimulation, embryology part, and genetics. Since biopsy is performed only on good quality blastocysts, single embryo transfer is preferred. The confirmatory prenatal diagnostic testing is still recommended following a PGT-M, and to a lesser extent of PGT-SR, due to difficulties of testing the limited number of cells obtained by embryo biopsy as well as recognition of the biological and human factors that may lead to misdiagnosis in a PGT cycle (6). A follow-up of pregnancies, deliveries, and postnatal development of born children, along with the cycle data, is maintained.

For cycles from 2004 to the end of 2016, cleavage-stage embryo biopsy was performed on day three after fertilization, and two blastomeres, when possible, were withdrawn. Then according to the indication, either fluorescent in situ hybridization (FISH) analysis or polymerase chain reaction (PCR) based protocol were performed. The FISH based protocol consisted of set-up with probe selection and pre-cycle work-up on peripheral blood lymphocytes from both reproductive partners. FISH was carried out according to standard protocol using commercially available probes by Abbott Vysis, Cytocell, or Agilent SureFISH, and guidelines and recommendations by ESHRE (7). The turnaround time was 48 hours, which allowed for fresh embryo transfer on day five. PCR based protocol for single-gene disorders was performed according to guidelines and recommendations by ESHRE (8). PGT set-up included indirect analysis and direct genotyping, if appropriate (8).

In 2017, blastocyst biopsy (trophectoderm biopsy-TE) on days 5 to 7 was introduced. This allowed for whole genome amplification and next-generation sequencing (NGS) based 24-chromosome screening for chromosome and segmental abnormalities with a resolution of 10-20 Mb. Another advantage of this approach is that a pre-diagnostic set up is usually not required. The NGS-based protocol was carried out according to the manufacturer's recommendations (VeriSeq PGS, Illumina). In addition, genetic testing for single-gene disorders was carried out as mentioned above.

We have reviewed the medical records from 2004 to 2019 at our institute to determine the proportion of couples with genetic indications that opted for preimplantation genetic testing and signed informed consent. Based on this data, we defined the proportion of Slovenian couples who would opt for PGT as a first genetic testing.

Data analysis

By first reviewing medical records, we estimated the proportion of couples that would opt for PGT. We retrospectively collected the referrals for all performed PGT-M, PGT-SR, and PGT-A cycles from 2004-2019. In addition, we calculated the average and median age of female partners enrolled in PGT. Then we analysed data of the PGT cycles regarding the referrals (PGT-M, PGT-SR, PGT-A or X-linked disorder), the type of embryo biopsy (blastomere biopsy in 2004-2016, blastocyst biopsy in 2017-2019) and genetic testing approach for chromosome rearrangements (FISH for chromosome rearrangements in 2004-2016, next-generation sequencing-based 24-chromosome screening in 2017-2019). Then, we compared the clinical outcomes in 2004-16 and 2017-19 using Chi-square statistics to test the clinical effectiveness of different PGT approaches.

RESULTS

Following genetic counselling, approximately 32% of couples would opt for PGT, either because of chromosome rearrangements (88/284) or monogenic disorder (110/333). Referrals of performed PGT cycles are presented in tables 1 and 2. Of a total of 211 couples, there were 110 for PGT-M (Table 1), 88 carriers of either simple reciprocal chromosomal translocation or carriers of a complex or cryptic chromosome rearrangement for PGT-SR (table 2). In addition, there were 10 couples with sex chromosome mosaicism and 3 couples with repeated aneuploid conception for PGT-A. The age of female partners engaged in our PGT program in selected years were as follows: average age of the females in the couples was 32.6 (25-38 years, median 33 years) in 2004-2016 and 33 years (24-39 years, median 33 years) in 2017-2019.

Data collection of our PGT program throughout the years 2004-2019 are presented in the tables 3 and 4. A total of 211 couples underwent 375 PGT cycles. The most frequent indications were single gene disorder, followed by chromosome rearrangement, with X-linked disease being the least represented. There were 263 embryo transfers, which resulted in 94 clinical pregnancies, while 16 pregnancies (16/94, 17%) ended in spontaneous miscarriage. Eighty-four unaffected children were born, examined by the paediatrician and geneticist. Embryo diagnosis was possible in 94% in the years 2004-2016 but dropped to 83% in years 2017-19. The diagnostic drop in the later years was mainly due to amplification failure or poor-quality biopsies.

We present the data for years 2004-2016 and 2017-19 separately because different biopsy and genetic testing methods were used.

Data from 2004-2016 for FISH analysis for chromosome rearrangements and multiplex PCR for monogenic disorders performed on blastomeres are collected in table 3. Altogether, the clinical pregnancy rate was 31% per embryo transfer. There were, on average, 4 embryos suitable for biopsy per cycle. The miscarriage rate was 20% (10/51). There were 8 twin pregnancies and one triple pregnancy. In addition, two cases of hyperstimulation were reported. There were 11 cycles with no PGT either because oocytes were not fertilized, embryo arrest or poor-quality blastocysts.

In 2017-19 we implemented TE biopsy and NGS based 24-chromosome screening for chromosome rearrangements and aneuploidy screening. The data are represented in table 4. Altogether, the clinical pregnancy rate was 43% per embryo transfer. There were, on average, 3 embryos suitable for biopsy per cycle. The miscarriage rate was 9% (4/43). There were no twin or triple pregnancies nor any cases of hyperstimulation reported. No cases of misdiagnosis were reported. There were 20 cycles with no PGT either because oocytes were not fertilized, there was embryo arrest or poor-quality blastocysts.

Table 1. List of monogenic disorders.

Disorder	Number of couples
Duchenne muscular dystrophy	6
Huntington Disease	15
Facioscapulohumeral dystrophy	3
Spinal Muscular Atrophy	5
GJB1 X -linked Charcot Marie Tooth	4
Charcot Marie Tooth disease I	6
Von Hippel Lindau syndrome	4
Retinoblastoma	2
Myotonic dystrophy 1	10
Cystic Fibrosis	3
Sandhoff disease	2
Alport syndrome	4
Haemophilia A	6
IL1RAPL1 intellectual disability	2
Fragile X syndrome	3
Incontinentia pigmenti	2
ARPKD	2
Fabry disease	2
Other*	29

*Includes only one couple for each referral: Autosomal recessive deafness IA, Achondroplasia, WWOX encephalopathy, Glycine encephalopathy, Spondyloepiphyseal dysplasia congenita, Marfan syndrome, Neurofibromatosis I, Tuberous sclerosis I, Congenital adrenal hyperplasia, Alagille syndrome, Proliferative vasculopathy and hydranencephaly-hydrocephaly syndrome, Emery -Dreifuss Muscular dystrophy, Pachyonychia congenita, Metachromatic leukodystrophy, Fraser syndrome, Myofibrillar myopathy, Hypohydrotic ectodermal dysplasia, Schimke immunoosseous dysplasia, MED12 genopathy, Tavil Andersen syndrome, Norrie disease, Epidermolysis bulosa dystrophica, Adenomatous polyposis coli, MYH7-Hypertrophic cardiomyopathy, CDH1-cancer predisposition, FOXC1- Axenfeld-Rieger syndrome, RYR1 congenital neuromuscular disease.

Table 2. List of chromosomal rearrangements.

Translocation	Number of couples
45,XY,der13;14)(q10;q10)	8
45,XX,der(13;14)(q10;q10)	3
Simple reciprocal translocation male/ female carrier	71
47,XXY,t(12;22)(q12;q13.3) (5)/46,XY,t(12;22)(q12;q13.3)(45)	1
45,XX,der(15;20)(q10;q10), der(20;21)(p10;q10)	2
46,XX,t(11;18)(q23;q21).ish ins(11;18) (q21;q21.1q21.3)(WCP18+)	1
46,XX.ish t(X;17)(p22.1;p13.3)	1
46,XX.ish t(17;22)(q25.1;q13.33)	1

Table 3. Data collection 2004-2016.

Referral	XL disorder (sex selection)	PGT-A	PGT-SR	PGT-M	Total
Couples	6	10	48	55	119
Cycles (OR)	20	27	88	106	241
ET	19	30	56	58	163
Embryos for biopsy	87	122	453	364	1026
Diagnosis	81(93%)	109(89%)	442(97%)	332 (91%)	964 (94%)
Pregnancy	6	5	20	20	51
Miscarriage	0	2	2 (+2*)	6	10 (19.6%)
Children	7	3	19	16	45
	1x twins	N/A	1x twins, 1x triples	2x twins	4x twins, 1x triples
Deliveries	6	3	16	14	39
Pregnancy rate / ET (%)	32%	17%	36%	34%	31%
Delivery rate / ET (%)	32%	10%	29%	24%	24%
Cycles with no PGT	0	0	2	9	11

Legend: *- post amniocentesis.

Table 4. Data collection 2017-2019.

Referral	X-linked disorder (sex selection)	PGT-A	PGT-SR	PGT-M	Total
Couples	4	3	40	45	92
Cycles (OR)	5	6	49	74	134
ET	3	4	29	64	100
Embryos for biopsy	11	16	112	219	358
Diagnosis	10 (91%)	13 (81%)	91 (81%)	182 (83%)	296 (83%)
Pregnancy	2	2	13	26	43
Miscarriage	1	0	1	2	4 (9%)
Children	1	2	12	24	39
Deliveries	1	2	12	24	39
Pregnancy rate / ET (%)	N/A	N/A	45%	41%	43%
Delivery rate / ET (%)	33%	50%	38%	36%	37%
Cycles with no PGT	0	1	11	8	20

We compared the clinical outcome between both periods (2004-2016 versus 2017-19) using the Chi-square method with a p-value of less than 0.05 considered as significant. Implementation of blastocyst biopsy and chromosome-wide analysis significantly improved delivery rate per ET for chromosomal and monogenic indications in years 2017-19 (Chi-square 4.184, p= 0.03 and Chi-square 5.21, p= 0.02, respectively), while pregnancy rate per ET (Chi-square 3.08, p=0.07) was not statistically significant.

DISCUSSION

Our results of 15 years of experience show that PGT has become an established practice in addition to traditional prenatal diagnosis in Slovenia.

PGT is performed for requests associated with a high risk for a severe medical condition in offspring, either of chromosomal or monogenic origin. The most common referrals for PGT-SR were reciprocal translocation in female partners and Robertsonian translocation in male partners. By contrast, PGT-M was mainly requested for Huntington's disease, Duchenne muscular dystrophy, Haemophilia A, Myotonic dystrophy, Spinal muscular atrophy, and Charcot Marie Tooth disease. Furthermore, couples at high risk for adult-onset disorders or familial cancer predisposition presented 20% of all PGT-M referrals.

Our results are consistent with the published ESHRE PGT Consortium data collection (9, 10). Trophectoderm biopsy and genome-wide analysis increased the accuracy and reliability of the preimplantation genetic testing. When com-

paring the dataset from 2004-2016 to 2017-19, the delivery rates per embryo transfer significantly increased. The increase may be due to the substantial amount of starting material, whole genome amplification and genome-wide screening.

We observed that in 2004-2016, there were, on average, four embryos suitable for biopsy per cycle, while in 2017-2019, up to three embryos. This lower number was expected because, in 2004-2016, embryos were biopsied on day three at the cleavage stage and in 2017-2019 at the blastocyst stage, and not all cleavage stage embryos reached the blastocyst stage. Hormone stimulation may be associated with hyperstimulation syndrome, a life-threatening condition in the most severe form. Therefore, each patient's hormonal stimulation protocol in our clinic is adjusted to optimize follicle growth and avoid complications associated with hyperstimulation syndrome. Since 2017, only the freeze-all approach has been performed in PGT cycles, which is more convenient to prevent hyperstimulation than before, when fresh embryo transfers were performed. Putting the patients and their safety first is our priority as well as a critical indicator of the quality of a healthcare system, including IVF-PGT procedures.

Most PGT cycles in Slovenia were requested for PGT-M and PGT-SR (375 cycles, 91%). In addition, PGT-A cycles (33 cycles, 9%) were performed because of genetic indications, i.e., parental sex chromosome mosaicism, X-linked monogenic disorder or repeated aneuploid conception. In many IVF centres, PGT-A cycles predominate and are used to shorten the time to pregnancy in the treatment of infertile couples without genetic indication. In the recent ESHRE data collection (9), PGT-A comprised more than 60% of all reported procedures. PGT-A, as an extension of IVF, is not performed in our country nor in Denmark, France, Germany, Hungary, Lithuania, Norway, and the Netherlands (10, 11). However, embryo sex selection by PGT-A is allowed in some European countries to screen for X-linked diseases.

The monogenic referrals account for more than 50% of cycles and are increasing yearly. An increase in PGT-M is mainly due to improved genetic diagnostics by next-generation sequencing and preconception carrier screening.

The availability of PGT for couples with severe genetic indications represents a considerable reproductive option in Slovenia. The costs of PGT cycles are covered by the National Health Insurance, which allows equal access to health care services for eligible couples. Our national public healthcare system provides PGT services in accordance with the needs of the patients to ensure fair and accessible patient-centred medicine. Furthermore, following genetic counselling, about a third of couples at high risk of transmitting a genetic disease to their offspring would opt for the PGT procedure.

The practices of PGT vary across different jurisdictions and policy approaches, ranging from restrictive to permissive policy models (4). Countries may regulate PGT through state funding (Austria, Belgium, Germany, France, Italy, the Netherlands, Switzerland, United Kingdom, Sweden, Denmark, Finland, Canada), private (Australia, Israel, United States, Singapore, Brazil, Japan), or a mixture of the two models (Denmark, Finland, United Kingdom) (4). However, PGT practices change with time, according to technological development, diagnostic improvements, ethical considerations, and patient needs and demands.

There are certain limitations of our retrospective register-based study. First, we know that a small sample size (n=163 ET in 2004-2016 and n=100 ET in 2017-2019) represents a study limitation. Nevertheless, the clinical outcomes are comparable to larger centres and reflect the actual needs of our patients. It was expected that blastocyst biopsies would result in increased implantation and live-birth rates compared to blastomere biopsy (12); however, a large retrospective cohort study showed that a freeze-all strategy is beneficial in high responders but not in intermediate or low responders, thus refuting the idea that freeze-all cycles are preferable for all patients (13). Lastly, we neither addressed the clinical characteristics of the patients in terms of hormone levels, stimulation protocol, endometrium preparation, or the number of retrieved and matured oocytes nor whether socioeconomic status influences the decision regarding PGT. The study was focused on the development and provision of PGT services in our country rather than assessing the routine protocol for IVF-PGT procedure.

In conclusion, we report on our 15 years of experience in PGT, provided by the Slovenian healthcare system, where about a third of couples at risk for transmitting a severe genetic disorder to their offspring would opt for PGT. The results of our study show that the clinical outcomes of PGT cycles are comparable to other larger centres. Furthermore, our study demonstrates that PGT, when provided by the public healthcare system offering accessibility and equity, has become a considerable option in addition to traditional prenatal diagnosis.

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enc – coordinated PGT procedures. BPeterlin designed and supervised the study and revised the manuscript. All authors reviewed and approved the final manuscript.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content of writing of this article.

ETHICAL APPROVAL

This was register-based study where all the participants signed individual personal approval and permission before starting the treatment and did not have to be notified to the Ethics Committee according to Slovene law, (Personal Data Protection Act, Official Gazette of the Republic of Slovenia No 94/07, 2004). Additionally, by our law, we are obligated to collect data about assisted reproduction procedures and monitor the success rates (Healthcare Databases Act, Official Gazette of the Republic of Slovenia No 65/00, 2000; No 47/15, 2015; 31/18, 2018).

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