CASE REPORT

UDC: 575:618
DOI: https://doi.org/10.2298/VSP190715138T

Birth of a healthy child fathered by a man with Klinefelter's syndrome after preimplantation genetic testing

Rođenje zdravog deteta od oca sa Klajnfelterovim sindromom nakon preimplantacionog testiranja

Aleksandra Trninić-Pjević*†, Stevan Milatović*, Jelena Havrljenko‡, Ivana Kavecan*, Aleksandar Kopitović*

University of Novi Sad, *Faculty of Medicine, ‡Faculty of Physical Sciences and Mathematics, Department of Biology and Ecology, Novi Sad, Serbia; †Clinical Centre of Vojvodina, Department of Obstetrics and Gynecology, Centre for Reproductive Medicine, Novi Sad, Serbia

Abstract

Introduction. Most men with non-mosaic Klinefelter's syndrome (KS; 47, XXY) have azoospermia, and were until recently considered completely infertile. However, it has been confirmed that some non-mosaic patients had spermatozoa in the ejaculate, although severe oligoasthenoteratozoospermia was present in all of them. Although a high fertilization rate with an intracytoplasmic sperm injection (ICSI) procedure using sperm from a non-mosaic patient and the cumulative pregnancy rate following *in vitro* fertilization constitute 53%, the incidence of live births after ICSI using sperm of men with non-mosaic KS is very low: 1 in 8 cases. **Case report.** At the Clinical Centre of Vojvodina's Department of Gynecology and Obstetrics, a successful *in vitro* fertilization treatment was conducted using the sperm of a man with KS non-mosaic syndrome. Preimplantation genetic testing was performed for the embryo selection, and an euploid embryo was transferred in the subsequent natural cycle. Delivery of male newborn was spontaneous, vaginal, with head presentation, after 40 weeks and 3 days. The newborn's Apgar score was 10/10. His birth weight was 3,950 g, and his length was 55 cm. At 12 months of age, his psychomotor development was assessed as normal on the Brunet-Lézine psychomotor development scale. **Conclusion.** Due to an increased risk of chromosomal abnormalities (abnormalities of sex or autosomal chromosomes) in embryos from couples with KS males, preimplantation genetic testing is conducted to select a chromosomally normal embryo. This may help achieve pregnancy sooner, decrease the chance of multiple pregnancy and its potential complications, decrease the risk of miscarriage, and reduce the need for invasive prenatal diagnostic procedures and the risks of other complications, as well as the risk of late termination of pregnancy in case of pathological findings of the fetal karyotype using conventional diagnostic procedures.

Key words:
klinefelter syndrome; reproductive techniques, assisted; fertility; sperm injections, intracytoplasmatic; azoospermia, non-obstructive; preimplantation diagnosis.

Apstrakt

Uvod. Većina muškaraca sa nemozaičnim 47, XXY Klajnfelterovim sindromom (KS) su azoospermični i donedavno su smatrani potpuno neplodnima, sa izrazito nepovoljnom prognozom muškog fertiliteta. Međutim, potvrđeno je da neki muškarci sa nemozaičnim KS imaju spermatozoide u ejakulatu, mada je kod svih prisutan težak oblik oligoasthenoteratozoospermije. Uprkos visokoj stopi fertilizacije sa postupkom intracetoplazmatske injekcije spermatozoida (ICSI) korišćenjem spermatozoida muškaraca sa nemozaičnim KS, kao kumulativnoj stopi fertilizacije koja iznosi 53%, ipak incidencija živorođenja kod njih nakon ICSI veoma je niska i iznosi jedan od osam slučajeva. **Prikaz bolesnika.** Na Klinici za ginekologiju i akušerstvo Kliničkog centra Vojvodine u Novom Sadu uspešno je sproveden postupak vantelesne oplodnje spermom muškarca sa nemozaičnim KS, uz odabir embriona preimplantacionim genetskim testiranjem. Euploidni embrion prenesen je u matericu majke u njenom narednom, prirodnom ciklusu. Porođaj je bio spontani, vaginalni, prezentacija potiljačna, u 40 + 3 gestacijskoj nedelji. Rođen je zdrav dečak, telesne mase (TM) 3 950 g i telesne dužine (TD) 55 cm. Njegov Apgar skor (AS) iznosio je 10/10. U uzrastu od 12 meseci, na Brune-Lezin-ovoj skali psihomotornog razvoja dečak je pokazao uredan psihomotorni razvoj. **Zaključak.** Zbog povišenog rizika od nastanka hromozomskih aberacija kod embriona (ab

Correspondence to: Aleksandra Trninić-Pjević, University of Novi Sad, Faculty of Medicine, 21 000 Novi Sad, Serbia.
E-mail: aleksandra.trninic-pjevic@mf.uns.ac.rs

normalnosti na polnim ili autosomalnim hormozomima) kod parova gde muškarac ima Klajnefelterov sindrom, primenom preimplantacionog genetskog testiranja bira se jedan euploidan embrion, čime se skraćuje vreme do ostvarivanja trudnoće, smanjuje se verovatnoća spontanog pobačaja, verovatnoća višeplodne trudnoće i njenih eventualnih komplikacija, čime se smanjuje potreba za invazivnim prenatalnim dijagnostičkim procedurama i rizici eventualnih, njima izazvanih komplikacija, kao i rizici završetka trudnoće u kasnijoj gestaciji u slučaju patološkog nalaza kariotipa ploda klasičnim dijagnostičkim procedurama.

Ključne reči:
klajnfelterov sindrom; reprodukcija, asistirana, tehnike; plodnost; injekcije sperme, intracitoplazmatične; azoospermija, neopstruktivna; dijagnoza, preimplantacijska.

Introduction

Klinefelter's syndrome (KS) is one of the most common chromosomal aberrations of human sex chromosomes, often resulting in hypogonadism and male infertility – azoospermia or severe oligospermia. Accordingly, KS is one the most common genetic causes of male infertility, found in approximately 10% of all men who suffer from azoospermia. Epidemiological data show that KS has an estimated prevalence of between 1:400 and 1:1000 male births. The prevalence of KS was reported to be 0.1 to 0.2% in the general population and 0.15 to 0.17% in prenatally detected cases [1, 2].

Since KS may have variable phenotypic features, a large number of males remain undiagnosed until they are well into adulthood and have fertility problems. Nowadays, besides prenatal invasive procedures (chorionic villi sampling, amnio/cordocentesis), prenatal noninvasive testing is also available for detecting cell-free DNA in maternal blood in order to establish diagnosis of fetal chromosomal disorders in early pregnancy. In the Autonomous Province of Vojvodina, a high percentage of pregnant women (over 90%) and their partners choose the option of pregnancy termination when they receive prenatal diagnosis of KS and become familiar with its clinical presentation.

Azoospermia is diagnosed when no spermatozoa are detected upon microscopic evaluation (the absence of spermatozoa) in more than 90% of cases, although there is also a possibility of the emergence of oligospermia (low sperm count and motility), which provides a reasonable probability of parenthood by *in vitro* fertilization (IVF) [3].

At present, data in the literature show that spermatozoa can be found by a testicular sperm extraction technique (TESE) in about 40–50% of males with KS, with the incidence of pregnancy and live births in approximately 50% of cases [3].

Progress in the field of assisted reproduction techniques and the advent of intracytoplasmic sperm injection (ICSI) procedure improves the chances of normal fertilization and embryo development even in cases of oligozoospermia and azoospermia. In spite of the pathological karyotype (47, XXY), the retrieved spermatozoa can be used for the ICSI procedure in males with KS, enabling genetic fatherhood.

Both normal fertilization of the ovum and normal development of the embryo, with subsequent pregnancy and childbirth, were achieved involving both mosaic and non-mosaic KS patients, after successful sperm retrieval using the TESE procedure [4].

According to the data published in the 1990s, the use of the fluorescent *in situ* hybridization technique (FISH), 2.09–2.7% hyperdiploid sperm was found in males with mosaic and non-mosaic KS [4, 5]. There is little chance of passing extra chromosomes on to their offspring. Accordingly, healthy children were born after the TESE procedure with ICSI in cases of patients with non-mosaic KS [4, 5]. Moreover, cases of embryos derived from KS patients who have a non-mosaic 47, XXY karyotype were published in that period.

Before accessing the procedure itself, it is necessary for the couples to be able to obtain all the necessary information, as well as to be explained that sperm retrieval is necessary, and subsequently proceed to IVF. Since there is a likelihood of the occurrence of a pathological fetal karyotype, with the possibility of other chromosomal abnormalities [2–5, 6], couples are given the option of choosing preimplantation genetic testing or other prenatal procedures for the diagnosis of chromosomal aberrations if conception occurs.

Preimplantation genetic testing (PGT) and preimplantation genetic diagnosis (PGD) were initially used for early biopsy and analysis of normal, unaffected embryos prior to their implantation into the mother's womb, with the aim of achieving a higher rate of successful implantations and live births of healthy children. Initially, the PGT/PGD method involved the use of FISH technique on polar bodies, in which biopsied blastomeres or trophectoderm cells were seeded on the cell culture plate. New methods have been developed, such as array comparative genome hybridization (aCGH), which is currently applied at the Clinical Centre of Vojvodina's Department of Gynecology and Obstetrics, and at the Centre for Medical Genetics at the Child and Youth Health Care Institute of Vojvodina, Novi Sad, in addition to the next generation sequencing (NGS). Clinical application of aCGH technique detects whether there are quantitative differences in the number of copies of a DNA sequence in a DNA sample of the analyzed embryo, as well as the likelihood of detecting aneuploidy and unbalanced rearrangements of chromosomes [7, 8, 12, 13]. Balanced chromosomal rearrangements (balanced translocations or inversions, etc.), in which the amount of genetic material does not change, may not be detectable by CGH.

Case report

A couple (a 23-year-old woman and a 33-year-old man) was presented at the Centre for Reproductive Medicine at the Clinical Centre of Vojvodina for IVF on an outpatient basis. The man was diagnosed with non-mosaic KS (47, XXY) through the G-banding technique.

Examination findings revealed azoospermia, an increased follicle-stimulating hormone (FSH) level of 18.1, and the testosterone level of 5.0 pg/mL.

In vitro fertilization was performed at the Department of Gynecology and Obstetrics. The analysis of the man's ejaculated spermatozoa revealed non-progressive, low sperm count, poor motility, and morphological irregularities (cryptozoospermia). Simultaneously, the woman underwent ovarian stimulation consistent with the antagonist protocol, using 200 IU rFSH (Puregon, MSD). Consequently, 9 oocytes were retrieved, from which 7 reached metaphase II (MII) status. Following the ICSI procedure, 4 blastocyst stage embryos were retrieved by trophotoctoderm biopsy for PGT.

PGT showed the presence of one euploid blastocyst, which was subsequently transferred to the woman's uterus during a natural cycle.

Pregnancy was confirmed by the amount of β-human chronic gonodatropin (β-hCG) serum in the blood. During pregnancy, the patient refused any further prenatal testing, although advised. The course of pregnancy was uneventful.

Delivery was spontaneous, vaginal, with head presentation, after 40 weeks and 3 days. A healthy boy was born. His Apgar score was 10/10, birth weight was 3,950 g and his length was 55 cm. At 12 months of age, his psychomotor development was assessed as normal on the Brunet-Lézine psychomotor development scale.

Methods

The blastocyst biopsy involves opening the zona pellucida on the fifth cultivation day at the opposite side of the inner cell mass (ICM). After several hours of cultivation, trophectoderm cells begin to hatch out of the zona pellucida through the hole, and they can be used for analysis.

First, the biopsied cells of the embryo were rinsed with the washing medium (manufactured by Origio), and placed into the phosphate-buffered saline (PBS + PBP) solution. Then they were placed into special sterile 0.2 mL microreaction PCR tubes**.** Sterilization was accomplished through exposure to UV light. Samples were placed into microtubes which had been previously filled with 200 μL PBS + PVP solution, with one sample put in each tube. In addition to the microtubes containing the samples, a control microtube was prepared, containing only the aforementioned solution. All microtubes were labelled, including the control microtube. The samples thus prepared were transferred to the Molecular Genetics Laboratory at the Children and Youth Health Care Institute of Vojvodina, Novi Sad. The transfer was performed under stringently controlled conditions.

Array comparative genomic hybridization testing was performed in the reference genetics laboratory. Each DNA sample was placed on a genomic chip-slide and immobilized (Illumina assay). Each slide consisted of large-insert genomic clones covering all autosomes and sex chromosomes at the range of approximately 100–200 kb, providing a detailed chromosomal analysis. The analysis was carried out using laser scanning software that displayed the results in the form of an algorithm. The whole procedure of the microovary-based aCGH analysis, from biopsy to outcome assessment, was completed within 16 hours. Thus, if blastocyst biopsy is performed (after five days of cultivation), it is necessary to perform embryo cryopreservation during the aCGH analysis, until euploid blastocyst stage embryo transfer can take place.

Discussion

First pregnancy outcomes after PGD were reported in 1990. They were performed with the aim of preventing transmission of X-linked diseases [9]. Since then, PGD has been implemented in couples with both structural and numerical aberrations or gene disorders, to avoid transmission of genetic disease to their offspring. PGD involves the biopsy of embryos, nowadays typically performed on trophectodermal cells biopsied from blastocysts, with removal of 8–10 blastomers at the blastocyst stage. The European Society of Human Reproduction and Embryology (PGD consortium) was established to monitor extra-uterine pregnancy outcomes by recording the number of babies born as a result of IVF with PGD, in addition to collecting data from centres throughout the world [10]. The follow-up of the children born after PGD or PGT has confirmed that their growth and development up to 2 years of age is similar to the children whose conception was natural. [11].

In general, in mosaic and non-mosaic KS patients, the possibility of sperm retrieval is about 30–50%, although predictive value for sperm retrieval is higher in mosaic KS [12].

A majority of men with non-mosaic KS (47, XXY) have azoospermia, and until recently they were considered completely infertile. However, it has been confirmed that some non-mosaic KS patients have spermatozoa in the ejaculate, although severe oligoasthenoteratozoospermia is present in all of them. A high fertilization rate with the ICSI procedure in mosaic KS was reported in 1995 [13].

The cumulative pregnancy rate following IVF constitutes 53% [14]. However, the incidence of live births after ICSI where fathers had non-mosaic KS is very low, and clinical pregnancy rates were recorded in 7 out of 10 cases [15–17]. It has been described earlier that only normal 46, XY cells can complete the meiotic process, and therefore every spermatozoid produced by a KS patient [18] has a high probability of having an abnormal karyotype. Unequal number of X chromosomes can lead to cell death [19]. Literature suggests, however, that meiotic progression and sperm production are possible in men with non-mosaic KS.

PGT is now additional practice aiming to help couples with KS men to achieve pregnancy sooner. According to literature, 54% of embryos derived from KS patients were found to be euploid [20]. Detected abnormalities refer to the

presence of an extra chromosome or the absence of a particular chromosome, observed in triploid or tetraploid embryos. The results of PGT show that sex chromosome abnormalities are present in about 15% of embryos, which is significantly higher than in healthy general population (only 3%). Therefore, compared to other embryos, embryos derived from KS males have a higher proportion of pathological findings. Namely, a higher proportion of sex chromosome disomy in patients with KS [21] was observed. The close proximity of the 21st chromosome to the sex chromosome during chromosome segregation, in the process of meiosis, may lead to the failure in segregation of chromosome 21 if the seminal vesicles are not palpable or they are not prominent – which is the case with KS [21]. After all, the benefit of PGT in couples with KS males is debatable: there is no indication that embryos derived from KS patients show a higher prevalence of sex-chromosome aneuploidy [20]. Furthermore, results in ICSI cycles comparing non-PGT cycles with PGT cycles did not show significant differences in implantation, clinical pregnancy, and live birth rates per cycle [21].

The frequency of chromosomal abnormalities in spermatozoa is higher in men with KS, but up to date clarification has not been provided as to whether these abnormalities are the result of constitutional chromosome aberrations or the consequences of using testicular sperm extraction. Due to the increased risk of chromosomal abnormalities in embryos (abnormalities of sex or autosomal chromosomes) from couples with KS males, the use of PGT involves selecting a chromosomally normal embryo. This can help achieve pregnancy sooner, decrease the chance of multiple pregnancy and its potential complications, decrease the risk of miscarriage, reduce the need for invasive prenatal diagnostic procedures and the risks of other complications, as well as the risk of late termination of pregnancy in case of pathological findings concerning the fetal karyotype, using conventional diagnostic procedures. The selection of chromosomally normal embryos, based on preimplantation genetic screening (PGS), eliminates the chance of transferring embryos carrying chromosomal aberrations. PGS/ICSI was performed in 26 treatment cycles involving KS patients, which resulted in 8 pregnancies [20]. Thus, information about the possibility of achieving pregnancy can be provided to couples with KS males, in addition to providing them with information about prenatal diagnosis of KS.

Although PGT is highly accurate, no test is 100% reliable, and this is due to the mosaicism of the embryo biopsied. Couples need to be counselled about the necessity of performing further non-invasive or invasive testing during pregnancy. Both PGT and non-invasive prenatal testing (NIPT) are associated with false positive and false negative results due to trophoblast-derived mosaicism. Mosacism is present when an embryo contains two or more distinct cell lines. The increased sensitivity of the next-generation sequencing (NGS) technology can recognize it in preimplantation embryos. Mosaicism is the result of mitotic errors during embryo development. With the next-generation sequencing (NGS), 10–20% of PGT are mosaic. This can cause a false positive or false negative PGT result [22–25].

The first trimester combined screening test is the gold standard for calculating the risk for trisomy 21, 13 and 18, with a detection rate of 95% when nuchal translucency, nasal bone, ductus venous, and tricuspid valve blood flow are assessed. The management plan for patients undergoing IVF with PGS should be first trimester screening, followed by comprehensive counselling and support, and recommendation of non-invasive prenatal testing or invasive testing, depending on the findings. The role of the IVF specialist is to recommend the right test for the right patient.

After achieving pregnancy, with or without PGT, it is always advisable to confirm the results of PGT through other non-invasive or invasive prenatal tests, with amniocentesis providing a high diagnostic certainty.

So far there has been no indication that embryo biopsy causes an increased risk of adverse neonatal outcome [30].

Conclusion

PGT of embryos from couples with KS males is recommended in order to achieve pregnancy sooner, and enhance the chances of success.

REFERENCES

1. *McKinlay Gardner RJ, Amor DJ.* Sex Chromosome Aneuploidy and Structural Rearrangement. In: *McKinlay, Gardner RJ, Sutherland GR, Shaffer LG*, editors. Chromosome Abnormalities and Genetic Counseling. 5th ed. Oxford: Oxford University Press; 2018.
2. *Bojesen A, Juul S, Gravholt CH.* Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab 2003; 88: 622–6.
3. *Corona G, Pizzocaro A, Lanfranco F, Garolla A, Pelliccione F, Vignozzi L,* et al. Sperm recovery and ICSI outcomes in Klinefelter syndrome: a systematic review and meta-analysis. Hum Reprod Update 2017; 23(3): 265–75.
4. *Palermo GD, Schlegel PN, Sills ES, Veeck LL, Zaninovic N, Menendez S,* et al. Births after intracytoplasmic injection of sperm obtained by testicular extraction from men with non-mosaic klinefelter's syndrome. N Engl J Med 1998; 388(9): 588–90.
5. *Ron-El R, Strassburger D, Gelman-Kohan S, Friedler S, Raziel A, Appelman Z.* A 47, XXY fetus conceived after ICSI of spermatozoa from a patient with non-mosaic Klinefelter's syndrome. Hum Reprod 2000; 15(8): 1804–6.
6. *Kahraman S, Findikli N, Berkil H, Bakircioglu E, Donmez E, Sertyel S,* et al. Results of preimplantation genetic diagnosis in patients with Klinefelter's syndrome. Reprod Biomed Online 2003; 7(3): 346–52.
7. *Fiorentino F.* Array comparative genomic hybridization: its role in preimplantation genetic diagnosis. Curr Opin Obstet Gynecol 2012: 24: 203–9.
8. *Munné S.* Preimplantation genetic diagnosis for aneuploidy and ranslocations using array comparative genomic hybridization. Curr Genomics 2012; 13(6): 463–70.
9. *Handyside AH, Kontogianni EH, Hardy K, Winston RM.* Pregnancies from biopsied human preimplantation embryos sexed by

Y-specific DNA amplification. Nature 1990; 344(6268): 768–70.

10. *De Rycke M*, *Goossens V*, *Kokkali G*, *Meijer-Hoogeveen M*, *Coonen E*, *Moutou C*. ESHRE PGD Consortium data collection XIV–XV: cycles from January 2011 to December 2012 with pregnancy follow-up to October 2013. Hum Reprod 2017; 32(10): 1974–94.
11. *Desmyttere S, Bonduelle M, Nekkebroeck J, Roelants M, Liebaers I, Schepper J*. Growth and health outcome of 102 2-year-old children conceived afterpreimplantation genetic diagnosis or screening. Early Hum Dev 2009; 85(12): 755–75.
12. *Westlander G, Ekerhovd E, Granberg S, Hanson L, Hanson C, Bergh C*. Testicular ultrasonography and extended chromosome analysis in men with nonmosaic Klinefelter syndrome: a prospective study of possible predictive factors for successful sperm recovery. Fertil Steril 2001; 75(6): 1102–5.
13. *Harari O, Gronow M, Bourne H, Johnston I, Baker G*. High fertilization rate with intracytoplasmic sperm injection in mosaic Klinefelter's syndrome. Hum. Reprod 1995; 63(1): 182–4.
14. *Kitamura M, Matsumiya K, Koga M, NishimuraK, Miura H, Tsuji T,* et al. Ejaculated spermatozoa in patients with non-mosaic Klinefelter's syndrome. Int J Urol 2000; 7(3): 88–92; discussion 93–4.
15. *Bourne H, Stern K, Clarke G, Pertile M, Speirs A, Baker HW*. Delivery of normal twins following the intracytoplasmic injection of spermatozoa from a patient with 47, XXY Klinefelter's syndrome. Hum Reprod 1997; 12(11): 2447–50.
16. *Tournaye H, Camus M, Vandervorst M, Nagy Z, Joris H, Van Steirteghem A,* et al. Surgical sperm retrieval for intracytoplasmic sperm injection. Int J Androl 1997; 20 Suppl 3: 69–73.
17. *Reubinoff BE, Abeliovich D, Werner M, Schenker JG, Safran A, Lewin A*. A birth in non-mosaic Klinefelter's syndrome after testicular fine needle aspiration, intracytoplasmic sperm injection and preimplantation genetic diagnosis. Hum Reprod 1998; 13(7): 1887–92.
18. *Ron-El R, Friedler S, Strassburger D, Komarovsky D, Schachter M, Raziel A*. Case report: Birth of a healthy neonate following the intracytoplasmic injection of testicular spermatozoa from a patient with Klinefelter's syndrome. Hum Reprod 1999; 14(2): 368–70.
19. *Skakkebaek NE, Philip J, Hammen R*. Meiotic chromosomes in Klinefelter's syndrome. Nature 1969; 221(5185): 1075–6.
20. *Staessen C, Tournaye H, Van Assche E, Michiels A, Van Landuyt L, Devroey P,* et al. PGD in 47, XXY Klinefelter's syndrome patients. Hum Reprod Update 2003; 9(4): 319–30.
21. *Muller J, Skakkebaek NE, Ratcliffe SG*. Quantified testicular histology in boys with sex chromosome abnormalities. Int J Androl 1995; 18(2): 57–62.
22. *Hennebicq S, Pelletier R, Bergues U, Rousseaux S*. Risk of trisomy 21 in offspring of patients with Klinefelter's syndrome. Lancet 2001; 357(9274): 2104–5.
23. *Victor AR, Tyndall JC, Brake AJ, Lepkowsky LT, Murphy AE, Griffin DK,* et al. One hundred mosaic embryos transferred prospectively in a single clinic: exploring when and why they result in healthy pregnancies. Fertil Steril 2019; 111(2): 280–93.
24. *Neal SA, Morin SJ, Franasiak JM, Goodman LR, Juneau CR, Forman EJ*. Preimplantation genetic testing for aneuploidy is cost-effective, shortens treatment time, and reduces the risk of failed embryo transfer and clinical miscarriage. Fertil Steril 2018; 110(5): 896–904.
25. *Lee E, Chambers GM, Hale L, Illingworth P, Wilton L*. Assisted reproductive technology (ART) cumulative live birth rates following preimplantation genetic diagnosis for aneuploidy (PGD-A) or morphological assessment of embryos: A cohort analysis. Aust N Z J Obstet Gynaecol 2018; 58(5): 525–32.
26. *Homer HA*. Preimplantation genetic testing for aneuploidy (PGT-A): The biology, the technology and the clinical outcomes. Aust N Z J Obstet Gynaecol 2019; 59(2): 317–24.
27. *PGT Consortium Steering committee. Carvalho F, Coonen E, Goossens V, Kokkali G, LLuesa CR, Meyer-Hoogeveen M,* et al. ESHRE PGT Consortium good practice 2 recommendations for the organisation of preimplantation genetic testing. 2019. Available from: http//www.eshre.eu>sitecore-files>Gudlines>PGT

Received on July 15, 2019
Revised on December 2, 2019
Accepted December 5, 2019
Online First December, 2019