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Please cite this article BIRTH OF A HEALTHY CHILD AFTER PREIMPLANTATION GENETIC TESTING IN A FATHER WITH KLINEFELTER'S SYNDROME IN SERBIA

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UDC:

DOI: https://doi.org/10.2298/VSP190715138T

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
BIRTH OF A HEALTHY CHILD AFTER PREIMPLANTATION GENETIC TESTING IN A FATHER WITH KLINEFELTER'S SYNDROME IN SERBIA

ROĐENJE ZDRAVOG DETETA OD OCA SA KLINFELTEROVIM SINDROMOM NAKON PREIMPLANTACIONOG TESTIRANJA

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Abstract

Introduction. The majority of Klinefelter syndrome cases with non-mosaic 47,XXY form have azoospemia and until recently they were considered completely infertile, with a markedly unfavorable and definitely poor male fertility prognosis. However, it has been confirmed that some non-mosaic patients have spermatozoa in the ejaculate deposition, although the form of severe oligasthenoteratospermia is present in all of them. Although high fertilization rate with ICSI procedure using sperm from a non-mosaic patient and cumulative pregnancy rate following in-vitro fertilization constitutes 53%, the incidence of live birth rate after ICSI in cases of non-mosaic Klinefelter syndrome is very low. Case report. At the Clinic for Gynecology and Obstetrics of the Clinical Center of Vojvodina a successful in vitro fertilisation treatment was conducted in Klinefelter non-mosaic male, with the use of preimplantation genetic testing for embryo selection, and euploid embryo was transfered in the subsequent natural cycle. A healthy boy was born, delivery was spontaneous, vaginal, with head presentation, after 40 weeks and 3 days, by which Apgar scores were: 10/10, birth weight was 3950g and length 55cm. The psychomotor development scale of Brunet-Lézine was used when the boy reached 12 months of age and he was assessed as having normal psychomotor development.

Conclusion. Due to the increased risk of chromosomal abnormalities in embryos (abnormalities of sex or autosomal chromosomes) from couples where a male partner is affected by Klinefelter syndrome, the use of preimplantation genetic testing involves selecting a chromosomally normal embryo which could shorten the time to the first live birth, decrease the risk of miscarriage and the chance of multiple pregnancy and its potential complications, reducing the need for invasive prenatal diagnostic procedures and the risks of some other complications, as well as the risk of late termination of pregnancy in case of pathological findings of the fetal karyotype using conventional diagnostics procedures.

Key words: klinefelter syndrome; assisted reproductive techniques; fertility; intra-cytoplasmic sperm injection; non-obstructive azoospermia; preimplantation diagnosis.
**Apstrakt**

**Uvod.** Većina slučajeva sa nemozaičnim 47, XXY Klinefelterovim sindromom su azoospermični i do skora su smatrani potpuno neplodnim, sa izrazito nepovoljnom i definitivno lošom prognozom muškog steriliteta. Međutim, potvrđeno je da neki nemozaični KS pacijenti imaju spermatozoide u ejakulatu, mada je kod svih prisutan težak oblik oligoastenotateratozoospermije. Uprkos visokoj stopi fertilizacije sa postupkom ICSI korišćenjem spermatozoida pacijenta sa nemozaičnim Klinefelterovim sindromom, kao kumulativnoj stopi fertilizacije koja iznosi 53% (19), ipak incidencija živorođenja kod njih nakon ICSI je veoma niska i iznosi jedan od osam slučajeva. (4,20,21,22,23).

**Prikaz slučaja.** Na Klinici za ginekologiju i akušerstvu Novom Sadu, Kliničkog centra Vojvodine uspešno je sproveden postupak vantelesne oplodnje kod nemozaičnog Klinefelter sindrom muškarca, uz selekciju embriona preimplantacionim genetskim testiranjem čime se euploidan embrion transferirao u narednom, prirodom, ciklusu žene. Rodjen je zdrav dečak, porođaj spontani, vaginalni, prezentacija potiljačna, u 40 +3 gestacijskoj nedelji, AS:10/10, TM 3950g i TD 55cm. U uzrastu 12 meseci, procenom skale psihomotornog razvoja (Brunet-Lezine skala) dečak pokazuje uredan psihomotorni razvoj.

**Zaključak.** Zbog povišenog rizika od nastanka hromozomskih aberacija kod embriona (abnormalnosti na polnim ili autosomalnim hormozomima) od supružnika gde je muškarac Klinefelter sindrom, primenom preimplantacionog genetskog testiranja se selektuje jedan zdrav embrion čime se skraćuje vreme do ostvarivanje trudnoće, smanjuje verovatnoća spontanog pobačaja, smanjuje verovatnoća višeplodne trudnoće i potencijalnih komplikacija, čime se smanjuje potreba za invazivnom prenatalnim dijagnostičkim procedurama i rizici vezani za komplikacije u vezi sa njima, kao i rizici terminacije trudnoće u kasnijoj gestaciji u slučaju patološkog nalaza kariotipa ploda klasičnim dijagnostičkim procedurama.

**Ključne reči:**
klinefelterov sindrom; asistirane reproduktivne tehnologije; plodnost; intra-citoplasmatska sperm injekcija; neobstrukтивna azoospermia; preimplantaciona dijagnoza.
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 Klinefelter’s syndrome has an estimated prevalence of between 1:400 and 1:1000 male births. The prevalence of Klinefelter’s syndrome 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 Klinefelter’s syndrome may have a 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), there is a noninvasive testing used in prenatal diagnostics for detecting cell-free DNA in maternal blood in order to establish diagnosis of fetal chromosomal disorders in an early pregnancy. In the Autonomous Province of Vojvodina, a high percentage of pregnant women (over 90%) and their partners choose an option of pregnancy termination after they receive prenatal diagnosis of KS and become familiar with the clinical presentation and the diagnosis of KS.

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 fertilisation (3).

At present, data available in the literature demonstrate that spermatozoa can be found by TESE technique (testicular sperm extraction) in about 40-50% of males with KS, with the incidence of pregnancy and live birth rates achieved in approximately 50% (3).

Progress in the field of assisted reproduction techniques and the advent of intracytoplasmic sperm injection (ICSI) improves the chances for normal fertilization and embryo development in men with severe 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, which can be used to enable genetic fatherhood.
Both normal fertilization of the ovum and normal development of the embryo, with subsequent pregnancy and childbirth were achieved in both mosaic and non-mosaic KS patients, after successful sperm retrievals using the TESE procedure (4). According to the publications of the 90’, the use of fluorescent in situ hybridization technique (FISH), 2.09-2.7% hyperdiploid sperm were found in mosaic and non-mosaic males with KS (4.5). There is a little chance of passing extra chromosomes on to their offspring. Accordingly, healthy children were born after the TESE procedure with ICSI in cases of non-mosaic KS patients. (4.5). Moreover, cases of embryos conceived in KS patients who have a non-mosaic 47, XXY karyotype were published during 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 show that sperm retrieval is necessary, and subsequently proceed to the procedure of in vitro fertilization. Since there is a likelihood of the occurrence of pathological fetal karyotype, with the possibility of other chromosomal abnormalities (2-5,6), couples are given the possibility of choosing pre-implantation genetic diagnosis or other prenatal procedures for the diagnosis of chromosomal aberrations if conception occurs.

Pre-implantation genetic testing (PGT) and pre-implantation genetic diagnosis (PGD) emerged and became clinical practice used for early embryo biopsy and analysis prior to implantation of only normal, unaffected embryos into the mother's womb, with the aim of achieving a higher rate of implantation and live birth of a healthy child. Initially, PGD/T method implied 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, among which array comparative genome hybridization (aCGH), which is currently applied at the Clinic for Gynecology and Obstetrics of the Clinical Center of Vojvodina and at the Centre of Medical Genetics of the Institute for Child and Youth Health Care of Vojvodina, in Novi Sad, in addition to the next generation sequencing (NGS). Clinical application of aCGH technique determines 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 likelihood of detecting aneuploidy and unbalanced rearrangements of chromosomes (12, 13). Balanced chromosomal rearrangements (balanced translocations or
inversions, etc.), in which the amount of genetic material does not change, may not be able to detect using a CGH.

**Case Report**

The couple (a 23-year-old woman and a 33-year–old man) visited the Center for Reproductive Medicine of the Clinical Center of Vojvodina for the purpose of going through in vitro fertilization procedure on an outpatient basis. In its classic form, non-mosaic 47, XXY Klinefelter's syndrome was detected in the partner using the G-banding technique.

According to the available findings, the man presented azoospermia, increased FSH level of 18.1 and testosterone level of 5.0 pg/ml.

At the Department of Gynecology and Obstetrics, the procedure of in vivo fertilization was performed after the evaluation of partner's ejaculated spermatozoa revealing non-progressive, low sperm count, poor motility and morphological irregularities (Cryptozoospermia). Simultaneously the wife underwent ovarian stimulation consistent with the antagonist protocol, using 200 IU rFSH (Puregon, MSD), and consequently 9 oocytes were retrieved, from which 7 reached metaphase II (MII) status. Following the ICSI procedure, 4 (four) blastocysts - embryos were retrieved by performing trophotocoderm biopsy for the purpose of preimplantation genetic testing.

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

Pregnancy was verified by the amount of β-hCG serum present in the blood. During pregnancy, patient refused any further prenatal testing, although advised. Pregnancy went well with no complications.

Delivery was spontaneous, vaginal, with head presentation, after 40 weeks and 3 days, by which Apgar scores were: 10/10 and a healthy baby boy was born and his birth weight was 3950g and length 55cm. The psychomotor development scale of Brunet-Lézine was used when the boy reached 12 months of age and he was assessed as having normal psychomotor development.
**Methodology**

The biopsy technique of the blastocyst meant that the zona pellucida is opened at the fifth cultivation day at the opposite side of inner cell mass (ICM). After several hours of cultivation, trophicoderma cells began to hatch out of the zona pellucida through the hole, so they could be used for analysis.

The biopsied cells of the embryo were primarily rinsed with the washing medium (manufactured by Origio), and then moved into the PBS + PBP solution. Further, they were loaded into special, sterile PCR, 0.2 ml, clean micro-reaction tubes. Sterilization was accomplished through exposure to UV light. Samples were placed into microtubes which had been previously prepared with PBS + PVP solution with 200 µL, with one sample added to each tube. Also, in addition to the microtubes with the samples, a control microtube was prepared, which contained only the above mentioned solution. All microtubes had to be labeled, and the last served as a control microtube. Thus prepared samples were transferred to the Molecular Genetics Laboratory of the Institute for Children and Youth Health Care of Vojvodina in Novi Sad. Transfer was performed under highly controlled conditions.

Array CGH testing was performed in the reference genetics laboratory. When performing testing, each DNA sample was placed on a genomic chip-slide – DNA immobilized on the glass (Illumina assay). Each slide consisted of large-insert genomic clones covering all autosomes and sex chromosomes at approximately 100–200 kb size range providing a more detailed chromosomal analysis. The analysis was carried out using laser scanning software that displayed the result represented in the form of an algorithm. The whole procedure of the aCGH analysis, from the biopsy procedure 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 process until the moment of embryo transfer of eupoidal blastocysts.
Discussion

First subsequent pregnancy outcomes after PGD were reported in 1990 and they were performed with the aim of preventing the transmission of X-linked diseases (14). Since then, PGD has been implemented in couples with both structural and numerical aberrations or gene disorders to avoid the transmission of genetic disease in their offsprings. PGD implies the biopsy of embryos, today typically performed on trophectodermal cells biopsied from blastocysts, with removal of 8-10 blastomers at blastocyst stage. The European Society of Human Reproduction and Embryology (PGD consortium) was established to monitor extra-uterine pregnancy outcomes by collecting the number of babies born as a result of in vitro fertilization with PGD, in addition to collecting data from centers throughout the world (15). The follow up of the children born after PGD or PGT has been confirmed with similar growth and development up to 2 years of age compared to normal conception (16).

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 (17).

For successful in vitro fertilization it is crucial to retrieve sperm, either by evaluating deposition of an ejaculate semen or by testicular biopsy. It is also noteworthy to mention that spermatozoids derived from testicular tissue can be cryopreserved for later use but there is a risk of about 20% that the retrieved sperm counts will not survive defrosting. That is why testicular biopsy is repeated on the day of oocyte retrieval, although it must be emphasized that it is sometimes technically difficult due to the small-size-testis.

The majority of KS cases with non-mosaic 47,XXY Klinefelter’s syndrome have azoospermia and until recently they were considered completely infertile, with a markedly unfavorable and definitely poor male fertility prognosis. However, it has been confirmed that some non-mosaic KS patients have spermatozoa in the ejaculate deposition, although the form of severe oligasthenoteratosoospermia is present in all of them. A high fertilization rate with ICSI procedure using sperm from a patient with non-mosaic KS was published in 1995. (18).

Cumulative pregnancy rate following in-vitro fertilization constitutes 53% (19). However, the incidence of live birth rate after ICSI in cases of non-mosaic Klinefelter
syndrome is very low and clinical pregnancy rates were detected in 7 out of 10 cases. (4,20,21,22,23)

It has been described earlier that only normal 46, XY cells can complete the meiotic process, and therefore every spermatozoid produced by KS patients (24) has a high probability of having an abnormal karyotype. The inequality of number of X chromosomes can lead to cell death (25). Literature citations suggest that meiotic progression is possible as well as sperm production in males with non-mosaic KS.

The use of PGT is now additional practice for couples with KS in order to shorten time to pregnancy. According to the available literature data, 54% of embryos from KS patients were found to be euploid and the study included 113 embryos of couples at different age in which the male suffers from Klinefelter's syndrome, which is less than in normal population constituting 77 %, evenly represented in both males and females (7). Detected abnormalities refer to the presence of an extra chromosome or missing of a particular chromosome, observed in triploid or tetraploid embryos. The results of pre-implantation genetic testing show that sex chromosome abnormalities are present in about 15% of embryos, which is significantly higher than in the healthy general population (only 3%). Therefore, compared to other embryos, embryos of KS parents have a higher proportion of pathological findings. Namely, a higher proportion of sex chromosome disomy in patients with KS (26) 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 (26). 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 (7). Furthermore, results in ICSI cycles comparing non-PGT cycles with PGT cycles did not show significant differences in implantation, clinical pregnancy and live birth rate per cycle according to Vloeberghs (26).

The frequency of chromosomal abnormalities in spermatozoa is higher in men with KS, but up to date clarification has not been provided 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 where a male partner is affected by KS, the use of PGS involves selecting a chromosomally normal embryo which could shorten the time to the first live birth, decrease the risk of miscarriage and the chance of multiple pregnancy and its potential complications, reducing the need for invasive prenatal diagnostic procedures and the risks of some other complications, as well as the risk of late termination of pregnancy in case of pathological findings of the fetal karyotype using conventional diagnostics procedures. The transfer of chromosomally normal embryos, due to the medical application of preimplantation genetic screening, eliminates the chance of transferring embryos carrying chromosomal aberrations. PGT / ICSI was performed in 26 treatment cycles with KS parent that resulted in 8 pregnancies (7). Thus, the information about the possibility of achieving pregnancy can be provided to couples with this condition in addition to providing them with information about a prenatal diagnosis of KS.

Although PGT is highly accurate, no test is 100% true, and that 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 in pregnancy. Both PGT and NIPT (Non Invasive Prenatal Testing) are associated with false positive and false negative results due to trophoblast-derived mosaicism. Mosacism is understood as an embryo containing two or more distinct cell lines. The increased sensitivity of next-generation sequencing technology (NGS) recognised it in preimplantation embryos. Mosaicisam is a result of mitotic errors during embryo development. With NGS 10-20% of PGT are mosaic. This can cause a false positive or false negative PGT result. (27-30).

First trimester combined screening test has been the gold standard for calculation of the risk for Trisomy 21, 13 and 18 with a detection rate of 95% when nuchal translucency, nasal bone, ductus venosus and tricuspid valve blood flow are assessed. The management plan for IVF patient with PGT should be first trimester screening test, followed by comprehensive counselling and reassurance or recommendation for NIPT or invasive testing depending on the findings. The role of the IVF specialist is to recommend the correct test for the correct patient.
After achieving pregnancy, with or without PGT, it is always advisable to confirm the results of PGT with different non-invasive or invasive prenatal tests, of which amniocentesis provides for higher diagnostic certainty.

So far there is no indication that embryo biopsy causes an increased risk for adverse neonatal outcome. (31).

**Conclusion**

PGT is suggested part of the analysis of embryos from the couples in which a male is affected by Klinefelter syndrome in order to shorten time to pregnancy and enhance chances to success.

Rad je realizovan zahvaljujući nabavci opreme za preimplantaciono genetsko testiranje i edukaciji embriologa Klinike za ginekologiju i akušerstvo Kliničko centra Vojvodine u okviru Posebnog programa zdravstvene zaštite pod nazivom “PROGRAM UNAPREĐENJA LEĆENJA STERILITETA BIOMEDICINSKI POTPOMOGNUTIM OPOLOĐENJEM I PREIMPLANTACIONOM GENETSKOM DIJAGNOSTIKOM NA TERITORIJI AUTONOMNE POKRAJINE VOJVODINE” koji je pokrenut 2013. godine (Službeni list APV “41/13).

This article is a result of equipment and educational support through Special health care program in Vojvodina “Improvement of infertility treatment with assisted reproductive technology and preimplantation genetic testing in Vojvodina”, which started in 2013. (Službeni list APV “41/13).

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