CASE REPORT OLGU SUNUMU

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Tetraploid Pregnancy Following a Frozen Thawed Embryo Transfer in an Intracytoplasmic Sperm Injection Cycle

ICSI Sonrası Dondurulmuş Çözdürülmüş Embriyo Transferi Sonrası Gerçekleşmiş Tetraploid Gebelik

Ulun ULUĞ[a], Oya ŞAHİN[b], Setenay ÜÇOK[b], Işıl UZUN ÇİLİNGİR[a]

[a]Department of Obstetrics and Gynecology, Haliç University Faculty of Medicine, İstanbul, Türkiye
[b]İstanbul Florence Nightingale Hospital, İstanbul, Türkiye

ABSTRACT

Here we report a case of a couple with primary infertility due to male factor. Gynecologic examination, basal hormone levels, and hysterosalpingography of the spouse were within normal limits. Sperm analyses were reported as severe oligoasthenoteratospermia. Peripheral blood karyotype analyses of the couple were normal. Controlled ovarian hyperstimulation (COH) with gonadotropins and ICSI were performed. A single frozen thawed blastocyst stage as 4AB quality embryo was transferred. The bhCG value on the 12th day after the transfer was 444 mIU/L. Gestational sac were observed on transvaginal ultrasound on the 22nd day following embryo transfer. At the following exams, embryo heartbeat was not detected and missed abortus was diagnosed. Pregnancy evacuation was performed during the 9th week of the pregnancy, and materials were separated for karyotyping. Cytogenetic evaluation of the conceptus was reported as tetraploidy (92,XXYY). Chromosomal segregation anomalies as mitotic failure following zygote stage can be considered for the development of multiploid embryos among ICSI cycle.

Keywords: ICSI; tetraploidy

ÖZET

Burada erkek faktörüne bağlı primer infertiliteye sahip bir çift olgusunu sunuyoruz. Eşin jinekolojik muayenesi, bazal hormon düzeyleri ve histerosalpingografisi normal sınırlardaydı. Sperm analizleri ciddi oligoasthenoteratatospermi olarak rapor edildi. Çiftin periferik kan karyotip analizleri normaldi. Gonadotropinler ve ICSI ile kontrollü over hiperstimülasyonu (COH) uygulandı. 4AB kalite embriyo olarak tek donmuş çözülmüş blastosist aşaması transfer edildi. Transfer sonrası 12. gün bhCG değeri 444 mIU/L idi. Embriyo transferi sonrası 22. günde yapılan transvajinal ultrasonda gebelik kesesi görüldü. Sonraki tetkiklerde embriyo kalp atışı saptanmadı ve miss abortus teşhisi konuldu. Gebeliğin 9. haftasında gebelik tahliyesi yapıldı ve karyotipleme için materyaller ayrıldı. Konseptusun sitogenetik değerlendirmesi tetraploidi (92,XXYY) olarak rapor edildi. Zigot aşamasını takiben mitotik başarısızlık gibi kromozomal ayrışma anomalileri, ICSI döngüsü arasında multiploid embriyoların gelişimi için düşünülebilir.

Anahtar Kelimeler: ICSI; Tetraploidi

Assisted reproductive treatment methods (ART) have become very common around the world in the last 20 years, and 1 out of every 10 babies born in developed countries is due to ART.[1] ART, which was an ideal method for tubal factor patients at the beginning, started to be applied in couples with a severe male factor with the introduction of intracytoplasmic sperm injection (ICSI) into practical laboratory use, and satisfactory results were obtained.[2] In vitro fertilization is performed as the classical method of ART. In this context, approximately 100000 spermatozoa are left around the oocytes under laboratory

Correspondence: ULUN ULUĞ
Department of Obstetrics and Gynecology, Haliç University Faculty of Medicine, İstanbul, Türkiye
E-mail: uulug@hotmail.com

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conditions and fertilization is expected to occur. In the ICSI method, a single spermatozoon is injected into the cytoplasm of the oocyte under a microscope. From this point of view, while there is a possibility of multispermic fertilization during IVF, this possibility has been eliminated in ICSI.

Multiploid gestations such as triploidy and tetraploidy karyotypes are encountered in abortus materials. It is also possible that some cases continue until the 2nd trimester of pregnancy. It even causes gestational trophoblastic diseases.[3] It may be of paternal or maternal origin.[4] During ART, the first 5 days of life are followed and fertilized embryos with any defect are not transferred. Since oocyte morphology and pronuclear morphology are always considered, embryos that may be polyploid are immediately recognized. During the fertilization phase, tripronuclear zygotes are observed. Since tripronuclear zygotes cause faulty cleavage, it is not preferred to be followed or its transfer is not considered appropriate. However, pregnancies resulting in a transfer of tripronuclear zygotes have been also described.

Tetraploidy has been described in spontaneous abortions or preimplantation embryos resulting from spontaneous conception or in vitro fertilization. However, to our best knowledge tetraploidy in early aborted fetuses resulting from ICSI has not been presented.

CASE REPORT

A 35-year-old female and a 39-year-old male couple applied with the complaint of 10 years of primary infertility. A gynecological examination revealed a retroverted uterus and free adnexal areas. In transvaginal ultrasonography, bilateral normal ovaries with >5 basal antral follicles with a normal-appearing endometrium were seen. Basal hormone levels (FSH, LH, TSH, AMH, and Prolactin) were within normal limits. Hysterosalpingography showed the uterus in normal appearance and passage. The couple did not have a history of chronic disease or previous surgical procedures. Repeated sperm analyzes were reported as severe oligoasthenoteratospermia (OAT). Peripheral blood karyotype analyzes of the couple were reported as normal. No Y chromosome microdeletion was detected in the spouse. Controlled ovarian hyperstimulation (COH) and ICSI were planned. As a result of the short flexible GnRH antagonist protocol, 20 oocytes were collected. ICSI was applied to 17 oocytes with metaphase 2. Single step medium (Sage 1-step, Origio, China) for embryo culture was utilized in bench top incubator (Miri, Esco,Lithuania). Two pronuclear zygotes were observed at 11. Eight cell grade 1, 6 embryos developed and 2 were transferred on Day 3. The remaining 4 embryos were cryopreserved with the vitrification method (Kitazato, freezing media, Japan). Neither of the embryos had assisted hatching with enzyme or laser. Frozen-thawed embryo transfer was planned 1 year later for the patient whose first embryo transfer result was negative. Endometrium preparation was performed with hormone replacement therapy. Before transfer, 4 embryos were thawed (Kitazato thawing media, Japan) and their development was followed for 2 more days. A single 4AB (Gardner blastocyst scoring) quality embryo that reached the blastocyst stage was transferred. The remaining embryos did not develop the blastocyst stage and therefore were arrested. Luteal phase support was performed with vaginal and intramuscular progesterone. The bhCG value on the 12th day after the transfer was reported as 444 mIU/L. A 15 mm gestational sac and a yolk sac were observed on transvaginal ultrasonography (TV USG) 22 days after embryo transfer. In the TV USG control performed two weeks later, it was found that the gestational sac enlarged, but the embryo body was not developed next to the yolk sac (Figure 1). A week later, no cardiac activity and any change of embryo body were detected so a missed abortion was diagnosed. According to the last menstrual period, pregnancy evacuation was performed under general anesthesia under USG guidance carefully aspirating embryonic material from the uterine cavity at the 9th week and the pregnancy material was separated for karyotyping. Abortion material was sent to genetic laboratory immediately. Pregnancy evacuation and postoperative period were uneventful. After cell culture for cytogenesis, the report was reported as tetraploidy (92, XXYY). During the period following surgical evac-

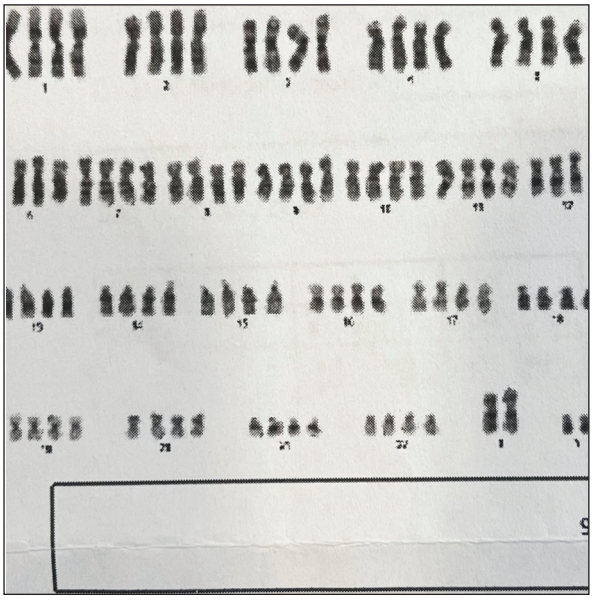

FIGURE 1: Karyotype analysis of the conceptus.

uation procedure serum bhCG levels were disappeared eventually.

DISCUSSION

To the best of our knowledge, a similar case has not been found in the relevant literature. There are illustrations of tetraploid embryos in assisted conception cycles, however, utilizing IVF or partial zona dissection (PZD).[5] Among those technologies, it is also probable that multispermic fertilization could occur. The most likely explanation for abnormally fertilized oocytes with 4PN after IVF and PZD is the penetration of three individual spermatozoa. In contrast to conventional IVF and PZD, the oocytes have to be freed from cumulus cells for ICSI and only mature female gametes in metaphase II (MII) are used further also oocyte maturity is judged by the presence of the first polar body (PB). Similarly, the suggested mechanism of molar pregnancies particularly incomplete moles is dispermic fertilization during natural conceptions. Furthermore, ICSI was recommended for individuals with repeated molar pregnancies.[6] Despite to the protective effect of ICSI, triploidy in an incomplete molar pregnancy was reported in an ICSI cycle by ours.[7]

Tetraploidy is a very rare finding in live-born infants. Tetraploid pregnancies in spontaneous conceptions have been reported. Phenotypes consist of prenatal and/or postnatal growth retardation, developmental delay, mental retardation, dysmorphic features, and skeletal and internal abnormalities.[8] Those fetuses usually die during the first days or months of life. Moreover, tetraploidy can be observed in 1%-2% of early spontaneous abortions.[9] Interestingly a case of a spontaneously conceived 26-month-old girl who had been diagnosed as tetraploidy with the karyotype of 92, XXXX resulted in dispermic fertilization of a diploid oocyte resulted by meiotic non-dysjunction was reported.[10]

Polyovularity and dispermic fertilization is the supposed mechanism of the development of tetraploid embryos in natural conception cycles. However, the data regarding the incidence of polyovularity and the significance of gametes originating from polyovular follicles are limited in the literature and the low number of cases complicates the ability to decide about the fertilization and implantation potential of these oocytes. The accepted definition of polyovular follicle is the inclusion of two oocytes within a common zona pellucida or their fusion in the zonal region and the most reliable reason for the formation of binovular follicles is a failure of separation of two individual germ cells in early folliculogenesis.[11] A recent case report showed that a genetically normal embryo can result from a con- joined oocyte, and subsequent pregnancy and live birth can be achieved.[12] It has been suggested also that tetraploidy may be produced by "concomitant fertilization of both meiotic products, that is the ovum and its second polar body, with a consequent fusion of the two resulting cells".[13] In a case report fertilization and development of embryos with conjoined zona pellucida recovered from polyovular follicles were reported.[14]

The fusion of two fertilized eggs is the second alternative mentioned by Guc-Scekic et al. Tetraploidy can arise from the fused two oocytes in a common zona pelucida.[10]

Moreover, tetraploidy caused by endoreduplication has been observed in a one-cell zygote and a two-cell embryo (15), suggesting that this process is a potential candidate for explaining some cases of polyploidy.[15] It is of note that certain cancer cells have aneuploidies and tetraploidy can also be seen among

them. In a study, centrosome duplication can result from extra chromosome gain and eventually lead to tetraploidy.[16]

On the other hand, it is out of consideration that binovular oocytes can be ignored during micromanipulation of gametes under a high magnified microscope. Therefore, it can be concluded that tetraploidy would occur following assisted fertilization. It should be encountered that during the ICSI procedure, it is imperative to inject more than one spermatozoon erroneously. Time lapse for karyotyping abortus material specimen could be important and long waiting time to sent specimens to laboratory could lead to false results.

Tripronuclear zygotes can be seen during assisted fertilization procedures even in ICSI cycles. Those embryos were discarded for embryo transfer for the possibility of abnormal implantation. Hence there are reports of healthy offspring following tripronuclear zygotes.[17] Furthermore, in assisted conception cycles triploidy is encountered in severe male factor infertility. The sperm centrosome could be responsible for the complex polyploid chromosome patterns observed in cleavage-stage embryos from an OAT patient.[18]

However, to our best knowledge, tetraploid pregnancy following the ICSI procedure has not been reported in the relevant literature. On the other hand, tetraploid embryos were demonstrated following assisted conception diagnosed by preimplantation genetic screening (PGS).

Preimplantation genetic analysis of embryos developed from unevenly cleaved zygotes revealed multinuclear tetraploid blastomeres, which points to a possible mitotic failure during embryogenesis.[19] Of note, the constitution XYYY that has never been reported in spontaneous abortions can be found at the zygote level.

In our case double Y chromosome could be derived by 2 approaches. One is erroneously injecting 2 spermatozoa in the same micropipette and both spermatozoa have a Y chromosome. Even in this case, there should be 2 nuclei and 2 polar bodies inside the zona pellucida which depicts a binuclear oocyte. This scenario is very far to be incident. The possible second mechanism of mitotic failure following ICSI. There could be a defect during anaphase to telophase in the mitotic cycle of blastomeres. It is speculative that in which phase of embryonic development, the cleavage stage or blastocyst stage, this defect occurred. It should be realized that if tetraploidy occurred following the blastocyst stage, a mosaic fetus could be possible. In our case, this mitotic error could be seen just after the zygote stage. During the cleavage stage from day 1 to 3, the nucleus of blastomeres can be inspected easily and the records of the patient do not reveal any abnormality. Therefore, either failure in karyokinesis and cytokinesis at the 4-cell stage or at the 2-cell stage followed by faithful 4:4 chromosome segregation may be the most likely explanation

The incubation period of embryos was spent on a benchtop incubator. We could speculate that if our case, for the incubation period, if a time-lapse embryo monitoring system was used, karyokinesis and cytokinesis during the cleavage period could be detected, and therefore a possible abnormality should be detected.

In conclusion, the presented case of tetraploidy is unique because of 3 factors. 1) following ICSI, 2) frozen-thawed embryo transfer, and 3) implantation resulting from a clinical pregnancy.

Source of Finance

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Ulun Uluğ;* ***Design:*** *Oya Şahin, Setenay Üçok;* ***Control/Supervision:*** *Işın Uzun Çilingir;* ***Data Collection and/or Processing:*** *Ulun Uluğ, Oya Şahin, Işın Uzun Çilingir;* ***Analysis and/or Interpretation:*** *Ulun Uluğ, Işın Uzun Çilingir;* ***Literature Review:*** *Ulun Uluğ, Işın Uzun Çilingir;* ***Writing the Article:*** *Ulun Uluğ, Işın Uzun Çilingir;* ***Critical Review:*** *Ulun Uluğ, Işın Uzun Çilingir;* ***Materials:*** *Oya Şahin, Setenay Üçok.*

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