

Pregnancy after the Calcium Ionophore Activation and Aneuploid Screening Using A-CGH in Globozoospermia Patient

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Abstract

Objective: To report a successful pregnancy after transfer of embryos derived from oocytes activated by calcium ionophore after intracytoplasmic sperm injection (ICSI) with round-headed sperms and aneuploid screening using a-CGH.

Design: Case report
Setting: Private IVF clinic.

Patient: A 28-year-old patients and her 31-year-old husband, diagnosed with 100% globozoospermia, underwent ICSI, oocyte activation and chromosome screening using a-CGH.

Intervention: Ten metaphase II oocytes were injected with round-headed sperms. After ICSI, the oocytes were treated with 10 µM calcium ionophore solution for 20 minutes at 37°C in 6% CO₂. The fertilization was checked 18 hours later. On morning of day 3, one blastomere was biopsied from embryos which had at least 6 cells and sent to the genetic lab for aneuploid screening using a-CGH. Two euploid embryos were transfer on the fifth day after oocyte retrieval. Supernumerary normal embryos were vitrified for future use.

Main Outcome Measure: Ongoing pregnancy after transfer of embryos after calcium ionophore activation and a-CGH screening.

Results: This couple experienced only 12% fertilization after ICSI in their first cycle. On the second cycle, 8 out of 10 metaphase II oocytes were fertilized after ICSI and calcium ionophore activation immediately after ICSI. 6 out of 8 embryos were diagnosed as euploid. Two normal blastocysts were chosen for transfer. Clinical pregnancy was confirmed at 7 weeks of gestation with two heartbeats. Two healthy babies were born.

Conclusion: Artificial oocyte activation using calcium ionophore is beneficial in patients with globozoospermia. This study showed that the method of oocyte activation does not affect chromosome constitution and the normal growth of preimplantation embryos. Further studies are needed to confirm the safety of oocyte activation in born babies.

Keywords: Globozoospermia; Round-headed sperm; Calcium ionophore; Oocyte activation.

Introduction

Globozoospermia is a rare type of teratozoospermia, first described in 1971. Biochemically, the spermatozoa are characterized by the absence, or reduced activity of acrosin (acrosome protease) and of calicin (a cytoskeletal protein) resulted in fertilization impaired due to absence of oocyte activation. The mode of inheritance for this condition has not yet been established. Dominant inheritance, homozygous autosomal gene defect, and a possible environmental effect have been suggested [2].

Affected men suffer from reduced fertility or even infertility; no other physical characteristics can be associated with this condition. An increased aneuploidy rate has been observed in some cases, on cytogenetic analysis, mostly in the acrocentric (13, 14, 15, 18 and 21)

and sex chromosomes but the findings are similar to other types of male infertility [3]. Increased DNA fragmentation and DNA damage has also been noted [4,5] The pathogenesis of globozoospermia most probably originates in spermiogenesis, especially in acrosome formation and sperm head elongation [2,6].

ICSI is the most powerful tool in ART. With the advent of ICSI, globozoospermia became one of the many severe male factor conditions that could be effectively treated. Although the fertilization rate with ICSI is considered to be the highest among all available assisted reproduction techniques, complete fertilization failure has been reported [7]. Ultra structural analysis of fertilization failure after ICSI has revealed that a deficiency in the mechanism of oocyte activation is the most common cause [1]. Activation of the oocyte results in a cascade of events including extrusion of the second polar body, decondensation of a haploid set of chromosomes, formation of a nuclear membrane around the chromosomes, and initiation of embryonic development. Oocyte activation is also characterized by

two main molecular events including an increase in intracellular Ca^{2+} concentrations followed by meiotic promoting factor inactivation for M-G1 transition. There are numerous approaches to overcome the problem of activation/fertilization failure after ICSI. Among others, application of calcium ionophores has proved particularly advantageous because of their ease of implementation. As exposure of human gametes to calcium ionophores has not yet been found to be associated with evidence of toxicity or detrimental outcome, its widespread use in cases of previous fertilization failure has become a routine technique. To date, no data on A23187 and gene expression are available. Although ionophores are not always successful [8], deliveries even in the presence of nonviable testicular spermatozoa are conceivable after treatment with A23187 [9]. Here we report the successful case of pregnancy and delivery in couples with globozoospermia after ICSI using calcium ionophore for oocyte activation, and pre implantation genetic screening for 23 pair chromosomes using array CGH.

Case Report

Infertility History

A couple with an infertility history of 2 years had gone through one IVF-ICSI cycle without oocyte activation with low fertilization rate (12%). The patient was 28 years old and was diagnosed with dysmenorrhea, dyspareunia and pelvic pain. The husband was 31 years old and his sperm revealed abundances of 20 Million/ml, 20% motility with all round-headed and acrosome less sperm.

Controlled ovarian stimulation

Patient was stimulated using Gonal F and Luveris and Antagonist. After 10 days of stimulation, 10 000 IU hCG was provided. 10 oocytes were retrieved by transvaginal follicle aspiration under ultrasound, 36 hours after hCG injection. The luteal phase was supported by crinone and estrace.

Intracytoplasmic sperm injection and assisted oocyte activation

Because all sperm were globozoospermic, those having the best motility were selected for injection. Right after ICSI, 10 oocytes were activated with calcium ionophore. For this activation oocytes were treated with 10 μ M calcium ionophore solution for 20 minutes at 37°C in 6% CO₂. After that the oocytes were washed three times with fresh culture medium until they were free of calcium ionophore and then incubated again in the same medium. The fertilization was evaluated after 18 hours after ICSI. 8/10 oocytes were fertilized.

Embryo biopsy and a-CGH

Fertilized oocytes were cultured until day 3. On the morning of day 3, six eight cells grade 1embryos and two six cells grade 1 embryo were biopsied. One blastomere was removed from each embryo and put in the PCR tubes and sent to Genetics Genesis lab for a-CGH evaluation. 6/8 embryos were euploid. 1 embryo was 45XX duplication of the short arm of chromosome 1 and monosomy 21; 1 embryo was aneuploidy monosomy 2;

Blastocyst culture and embryo transfer

On morning day 5, two normal hatching blastocyst with high quality were transferred to the uterus.

Outcomes

Clinical pregnancy was confirmed at 7 weeks of gestation with two heartbeats. Two healthy babies were born.

Discussion

Globozoospermia is an infrequent pathology in which spermatozoa lack acrosomes. Patients are considered sterile without IVF with ICSI treatment as spermatozoa with a characteristic round-head appearance and functionally with limited capacity to fertilize [10]. Before 1994, patients with complete globozoospermia have no options for bearing a child other than using donor sperm or adoption. In 1994, Lundin et al. reported the first pregnancy in globozoospermia patient using ICSI method [11]. Since then, numerous reports have described successful attempts to achieve either fertilization or pregnancy following ICSI with global sperm [12-24]. In general, ICSI with round-headed sperm is less successful compared with ICSI in general. In some cases ICSI is not always overcome the infertility associated with globozoospermia [25-27]. Some reports indicated that induction of oocyte activation using calcium ionophore was necessary after ICSI in some patients with globozoospermia [28-31], strongly pointing to an absence of putative oocyte-activation factor. In this case, using ICSI combine with calcium ionophore activation, we got fertilization rate of 80% comparable with general ICSI cases.

There are some reports that show a possible correlation between globozoospermia and chromosomal aneuploidies. Ditzel et al. observed that aneuploidy rates in spermatozoa from a patient with globozoospermia were significantly higher than in a normospermic man [32]. In that study they demonstrated a positive correlation between globozoospermia and higher chromosomal aneuploidies of chromosomes 13, 16, and 21. In our patient, the rate of aneuploidy was 2/8, and the chromosomes involved were 2, 16 and 21. The aneuploidy rate was lower than the common reported rate for women less than 35 year old of age undergoing IVF/ICSI cycle.

Our results support that artificial oocyte activation using calcium ionophore is beneficial in patients with globozoospermia. This study showed that the method of oocyte activation does not affect chromosome constitution and the normal growth of preimplantation embryos. Further studies are needed to confirm the safety of oocyte activation in born babies.

References

1. Kilani Z, Ismail R, Ghunaim S, Mohamed H, Hughes D, et al. (2004) Evaluation and treatment of familial globozoospermia in five brothers. *Fertil Steril* 82: 1436-1439.
2. Holstein AF, Schirren CG, Schirren C, Mauss J (1973) [Round headed spermatozoa: a cause of male infertility]. *Dtsch Med Wochenschr* 98: 61-62.
3. Machev N, Gosset P, Viville S (2005) Chromosome abnormalities in sperm from infertile men with normal somatic karyotypes: teratozoospermia. *Cytogenet Genome Res* 111: 352-357.
4. Vicari E, Perdichizzi A, De Palma A, Burrello N, D'Agata R, et al. (2002) Globozoospermia is associated with chromatin structure abnormalities: case report. *Hum Reprod* 17: 2128-2133.

5. Sutovsky P, Terada Y, Schatten G (2001) Ubiquitin-based sperm assay for the diagnosis of male factor infertility. *Hum Reprod* 16: 250-258.
6. Lalonde L, Langlais J, Antaki P, Chapdelaine A, Roberts KD, et al. (1988) Male infertility associated with round-headed acrosomeless spermatozoa. *Fertil Steril* 49: 316-321.
7. Moomjy M, Sills ES, Rosenwaks Z, Palermo GD (1998) Implications of complete fertilization failure after intracytoplasmic sperm injection for subsequent fertilization and reproductive outcome. *Hum Reprod* 13: 2212-2216.
8. Check JH, Levito MC, Summers-Chase D, Marmar J, Barci H (2007) A comparison of the efficacy of intracytoplasmic sperm injection (ICSI) using ejaculated sperm selected by high magnification versus ICSI with testicular sperm both followed by oocyte activation with calcium ionophore. *Clin Exp Obstet Gynecol* 34: 111-112.
9. Ahmady A, Michael E (2007) Successful pregnancy and delivery following intracytoplasmic injection of frozen-thawed nonviable testicular sperm and oocyte activation with calcium ionophore. *J Androl* 28: 13-14.
10. Dam AH, Feenstra I, Westphal JR, Ramos L, van Golde RJ, et al. (2007) Globozoospermia revisited. *Hum Reprod Update* 13: 63-75.
11. Lundin K, Sjögren A, Nilsson L, Hamberger L (1994) Fertilization and pregnancy after intracytoplasmic microinjection of acrosomeless spermatozoa. *Fertil Steril* 62: 1266-1267.
12. Bourne H, Liu DY, Clarke GN, Baker HW (1995) Normal fertilization and embryo development by intracytoplasmic sperm injection of round-headed acrosomeless sperm. *Fertil Steril* 63: 1329-1332.
13. Trokoudes KM, Danos N, Kalogirou L, Vlachou R, Lysiottis T, et al. (1995) Pregnancy with spermatozoa from a globozoospermic man after intracytoplasmic sperm injection treatment. *Hum Reprod* 10: 880-882.
14. Kilani ZM, Shaban MA, Ghunaim SD, Keilani SS, Dakkak AI (1998) Triplet pregnancy and delivery after intracytoplasmic injection of round-headed spermatozoa. *Hum Reprod* 13: 2177-2179.
15. Stone S, O'Mahony F, Khalaf Y, Taylor A, Braude P (2000) A normal livebirth after intracytoplasmic sperm injection for globozoospermia without assisted oocyte activation: case report. *Hum Reprod* 15: 139-141.
16. Coetzee K, Windt ML, Menkveld R, Kruger TF, Kitshoff M (2001) An intracytoplasmic sperm injection pregnancy with a globozoospermic male. *J Assist Reprod Genet* 18: 311-313.
17. Kim ST, Cha YB, Park JM, Gye MC (2001) Successful pregnancy and delivery from frozen-thawed embryos after intracytoplasmic sperm injection using round-headed spermatozoa and assisted oocyte activation in a globozoospermic patient with mosaic Down syndrome. *Fertil Steril* 75: 445-447.
18. Nardo LG, Sinatra F, Bartoloni G, Zafarana S, Nardo F (2002) Ultrastructural features and ICSI treatment of severe teratozoospermia: report of two human cases of globozoospermia. *Eur J Obstet Gynecol Reprod Biol* 104: 40-42.
19. Tesarik J, Rienzi L, Ubaldi F, Mendoza C, Greco E (2002) Use of a modified intracytoplasmic sperm injection technique to overcome sperm-borne and oocyte-borne oocyte activation failures. *Fertil Steril* 78: 619-624.
20. Zeyneloglu HB, Baltaci V, Duran HE, Erdemli E, Batioglu S (2002) Achievement of pregnancy in globozoospermia with Y chromosome microdeletion after ICSI. *Hum Reprod* 17: 1833-1836.
21. Heindryckx B, Van der Elst J, De Sutter P, Dhont M (2005) Treatment option for sperm- or oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI. *Hum Reprod* 20: 2237-2241.
22. Dirican EK, Isik A, Vicdan K, Sozen E, Suludere Z (2008) Clinical pregnancies and livebirths achieved by intracytoplasmic injection of round headed acrosomeless spermatozoa with and without oocyte activation in familial globozoospermia: case report. *Asian J Androl* 10: 332-336.
23. Yoon SY, Jellerette T, Salicioni AM, Lee HC, Yoo MS, et al. (2008) Human sperm devoid of PLC, zeta 1 fail to induce Ca(2+) release and are unable to initiate the first step of embryo development. *J Clin Invest* 118: 3671-3681.
24. Banker MR, Patel PM, Joshi BV, Shah PB, Goyal R (2009) Successful pregnancies and a live birth after intracytoplasmic sperm injection in globozoospermia. *J Hum Reprod Sci* 2: 81-82.
25. Liu J, Nagy Z, Joris H, Tournaye H, Devroey P, et al. (1995) Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia. *Hum Reprod* 10: 626-629.
26. Battaglia DE, Koehler JK, Klein NA, Tucker MJ (1997) Failure of oocyte activation after intracytoplasmic sperm injection using round-headed sperm. *Fertil Steril* 68: 118-122.
27. Rybouchkin AV, Van der Straeten F, Quatacker J, De Sutter P, Dhont M (1997) Fertilization and pregnancy after assisted oocyte activation and intracytoplasmic sperm injection in a case of round-headed sperm associated with deficient oocyte activation capacity. *Fertil Steril* 68: 1144-1147.
28. Rybouchkin A, Dozortsev D, Pelinck MJ, De Sutter P, Dhont M (1996) Analysis of the oocyte activating capacity and chromosomal complement of round-headed human spermatozoa by their injection into mouse oocytes. *Hum Reprod* 11: 2170-2175.
29. Gómez E, Pérez-Cano I, Amorochó B, Landeras J, Ballesteros A, et al. (2000) Effect of injected spermatozoa morphology on the outcome of intracytoplasmic sperm injection in humans. *Fertil Steril* 74: 842-843.
30. Schmiady H, Schulze W, Scheiber I, Pfuller B (2005) High rate of premature chromosome condensation in human oocytes following microinjection with round-headed sperm: case report. *Hum Reprod* 20: 1319-1323.
31. Tejera A, Molla M, Muriel L, Remohi J, Pellicer A, et al. (2008) Successful pregnancy and childbirth after intracytoplasmic sperm injection with calcium ionophore oocyte activation in a globozoospermic patient. *Fertility and Sterility* 90:1202.
32. Ditzel N, El-Danasouri I, Just W, Sterzik K (2005) Higher aneuploidy rates of chromosomes 13, 16, and 21 in a patient with globozoospermia. *Fertil Steril* 84: 217-218.