Outcome of vitrified oocyte injected by immotile testicular spermatozo with totally multiple morphological abnormalities of the sperm flagella :a case report

liuguang zhang[1], Yuhu Li[1], and Yuqun Huang[1]

[1]Affiliation not available

September 24, 2020

Outcome of vitrified oocyte injected by immotile testicular spermatozo with totally multiple morphological abnormalities of the sperm flagella :a case report

Introduction

In recent years,the terms of "MMAF" was more and more popular in the reserches about asthenozoospermia in male infertility, with a typical phenotype characterized by the presence of immotile sperm with cureled, short, bent, coiled, absent flflagellum or flflagellum with an irregular width[6,8,10-13,21,23,26,28,30,31]. Previous studies reported as dysplasia of the fifibrous sheath(DFS) or stump tails or non-specifific flflagellar anomalies[2,4,9,25,27]. Ben Khelifa M firstly described it as MMAF in 2014, a name that seems more accurate[3] . Electron microscopy examination of spermatozoa showed that MMAF are often accompanied by ultrastructural/functional defects of the axoneme, which contains 9 outer microtubules doublets and 2 central singlets (9+2 structure), several axonemal dyneins, radial spokes, nexin links and many other components[3,24]. Any defects in these structures would lead to abnormal sperm morphology of the flagella and further results in severe asthenozoospermia or 100% sperm immotility.

Another MMAF-like phenotype is observed in men with primary ciliary dyskinesia (PCD), which is determined by the presence of classical PCD Phenotypes such as recurrent respiratory tract infections, bronchitis, rhinosinusitis, bronchiectasis, and infertility[16].

At present,intracytoplasmic sperm injection (ICSI) is the best possible tool for MMAF patients to obtain healthy offspring.In previous MMAF cases,normaI fants delivered following ICSI were reported by other authors[7,21,19]. Here, we report pregnancy with the delivery of a girl after frozen embryo transfer from vitrified oocyte injected by immotile testicular spermatozo from a MMAF patient with no clinical features of PCD. This observation suggests that vitrified oocytes also could be fertilized by 100% immotility testicular spermatozoa with multiple morphological abnormalities of the sperm flagella,even with mostly curled end piece of the flagella.

Case presentation

The couple presented with 4 years of primary infertility. The 33-year-old woman had been assessed by a gynaecologist, and no contributing female factors were detected. After physical examination, the 34-year-old man was normal in the male reproductive system,and no significant respiratory symptoms were observed. His history provided no further information of clinical problems,and lymphocyte karyotype was 46, XY. Analysis of microdeletions of azoospermia factor genes on Y chromosome was normal.However,after repeated semen analyses by light microscope, the man was subjected to complete immotility and totally abnormal tail, namely MMAF with short, cureled, bent, coiled, absent flflagellum(Figure1A). According to the World Health Organization guidelines,Semen analyses showed sperm densities of 11-21 million/mL,

volumes of 2.6–3.6 mL, and sperm vitality of 39% with normal pH.The sperm flagellar displayed curled 64%, bent20%, short 7%, absent 3%, coiled 2%, irregular 4%(Table1). The couple had not previously attempted IVF.

A long protocol for ovulation induction was administrated by the daily administration of recombinant FSH (200 IU/day) following pituitary desensitisation with GnRH agonist. and cumulus-oocyte complexes were retrieved transvaginally 36 h later under ultrasound guidance after administration of 10,000 IU of human chorionic gonadotrophin (HCG). Oocytes were identified and maintained in culture under 6% CO2 in humidified air at 37°C .

After the oocyte retrieval procedure,cumulus-oocyte complexes were digested by using hyaluronidase (10 IU/ml). 22 of the 24 oocytes were confirmed as being at the metaphase II stage of meiosis . 10 mature oocytes were injected with ejaculated sperm and other 12 of them were vitrifed in the event of future ICSI attempts with testicular serpmatozoa. In the fresh cycle,6 of the 10 microinjected oocytes were fertilized with ejaculated sperm(Table1).On day 3 , two good quality embryos in culture were transferred. the other four non-good quality embryos did not develop to blastocyst after six days . Pregnancy was not achieved after 14 days of embryos' transfer.

Seven months later, the couple returned to the clinic in order to try again with their frozen oocytes and fresh testicular spermatozoa.Testicular spermatozoa were obtained by needleaspiration. Compared to ejaculated sperm,there was nothing changed about the testicular spermatozoa morphology,which showed that all were 100% immotile and curled or bent back on itself. Testicular sperm with comparatively normal morphology were injected to 12 warmed oocytes, which survied sucessfully after thawing. Nine two-pronuclei and one-pronuclei oocytes were obtained at 16 h post-ICSI.cleavage was observed in eight oocytes.on day 3,two 8-cell embryos with $< 20\%$ fragmentation were transferred.two of the remaining 6 embryos developed to blastocyst after D6,and a grade of 5BB blastocyst was frozened(Table1).Twelve days after transfer, the βHCG concentration was 80 mIU/dl. But a gestational sac with fetal heartbeat was not observed 3 weeks later.

Fortunately,offspring were obtained in the last attempt after transfer the frozen blastocyst of 5BB. A normal female baby was delivered,with a birth weight of 3050g and a length of 53cm.

Discusion

Recent years, more and more stdudies about MMAF had been reported, and normaI fants were delivered following ICSI with ejaculated sperm[6,8,10-13,21,23,26,28,30,31].To our knowledge , the present case is the first report of Successful birth after injection of vitrified oocyte by immotile testicular spermatozo with totally MMAF. This report shows that even in case of MMAF patient,vitrified oocytes can be successfully fertilizated by ICSI ,and produce healthy offspring.Thus,Vitrification may serve as a useful tool in the preservation of oocytes for MMAF couples .

Oocyte cryopreservation has become an important part of infertility treatment for various reasons.In our case,vitrification of a part of oocyte cohort was proposed to save oocytes for a potential subsequent ICSI cycle in case of no embryos available in the first cycle. Oocyte survival at the MII stage after warming, for this case report, was 100% (12/12 oocytes),similar to the post-warming survival rates of 96.4% with 86.2% fertilization rate in 2020 from Deepa Talreja[10]. In R Azambuja's report,it is 63% and 80% respectively[18]. While the fertilization rate in this report was 75% (9/12 surviving oocytes fertilized). This difference might be related to the different sperm origin, seriously impaired sperm motility of MMAF ;

According to the previos study, majority of the succesful MMAF cases are from the ejaculated sperm[1,3,5-8,14,15,22,23,25,27]. It is uncommon for MMAF patients that both the flagellar of the ejaculated sperm and testicular spermatoza are 100% abnormal and totally immotile.In Yang's report, testicular spermatozoa were obtained by needle aspiration,which were also showed 100% immotile,but with a small portion of nomal flagella. And the sperm flagellar phenotype was in accordance with MMAF,showing absent 20.0%, short38.5%, coiled 30.5%, irregular 5.5%, and bent 5.5%,respectively[21]. Robert l showed a PCD patient

that all testicular sperm were immotile,and the majority were structurally abnormal,being decapitated and with short thickened tails[19]. Similar to the above reports,both of the ejaculated and testicular spermatozoa were totally immotile and 100% abnomal flagella in our case(Figure1A). Different from them,Under light microscopy, 84% of the sperm flagellar phenotype were showing curled or bent back on itself at the end piece of the flagellar, but with normal head and middle piece percentage(Table1). To our knowledge, the case is the first report of MMAF that all the the morphology of the flagellar are abnormal,and majority of the end piece of the flagellar were cureled or bent.

The percentage of each abnormal sperm flagellar phenotypes was different in different MMAF patients.This difference might be related to the different disease-causing genes. Recent years, more and more new genes are reported to be associated with MMAF, including the latest DNAH8, DNAH17, CFAP61, QRICH2 and CFAP74[6,23,26,30,31]. These uncovered genes always lead to disrupted ultrastructural defects of the axoneme, which is the core structure of the sperm flagellar, several axonemal dyneins, radial spokes, nexin links and many other components. Any defects in these structures would result in abnormal sperm flagellar, showing a mosaic of morphological abnormalities, and further lead to severe asthenozoospermia or 100% sperm immotility.

The immotile spermatozoa in MMAF patients showed a viability of 9%–80%,and fertilization rate of 38.9%-75%[7,25]. These results are quite similar to those observed here. In the present case, with a Viability of 39%,the first ICSI cycle attempt with ejaculated sperm resulted in a 60% fertilization rate. While in the second cycle with fresh testicular spermatozoa,it was a better 75% fertilization rate.Yang's report showed a 45.5% fertilization rate,when testicular spermatozoa from a MMAF patient were injected into the oocytes[21]. Twelve of 18 oocytes were fertilized in another MMAF-like PCD patient after testicular sperm extraction and intracytoplasmic sperm injection[19].Our results further demonstrated that testicular sperm with MMAF are capable of establishing a normal pregnancy,regardless of also being immotile and totally abnormal . Moreover, maybe immotile testicular sperm is better than immotile ejaculated sperm and has been recommended for use rather than completely immotile ejaculated sperm in MMAF patients.

Addationally,This difference of fertilization rate might be related to the selection of viable sperms. The hypo-osmotic swelling test to evaluate the vitality of the spermatozoa prior to ICSI was not possible because of the totally abnormal end piece of the flagella. All of the spermatozoa in the papient exhibit curlings of the flagellum that resemble HOS-positive spermatozoa. This especially lilmited the efficency of the HOS test ,and the result would be inaccurate. In our case, morphologically and physical characteristics (tail flaccidity) were used to select sperm,accurately,the curled flagellar with normal head(Figure1B). A similar fertilization rate (67%) was achieved.This demonstrates that a high fertilization rate can be obtained even if HOS is not used for MMAF patients.

Interestingly, it was reported that the success rates of ICSI may be correlated to the type of ultrastructural flagellar defect carried by the patients. Mitchelletal reported lower implantation(8%) and clinical pregnancy rates(15%) in patients without axonemal centralpair[15] . Unfortunately,In the patient reported here,TEM was not done on the sample , so the ultrastructure of the spermatozoa is unknown.

The is no doubt that the finding genes provided strong genetic evidence for MMAF[17,20,22,26-31]. Thus, genetic counseling is highly significant in helping evaluate and avoid the risk of transmission of genetic defects by ICSI in these patients. In the present report, the chances of genetic transmission were lessened by the fact that the new-born was a girl . However, the possibility that this girl may be a healthy carrier of the described variant of the immotile cilia syndrome should be discussed during genetic counseling . We are sure that genetic counseling and careful analysis using electron microscopy will make the course of MMAF treatment better .

In conclusion, we firstly reported a sucessful delivery after injection of vitrified oocyte with immotile testicular spermatozo with MMAF. Although more and more new genes were revealed in recent years,these can only explain 35%-60% of the MMAF cases[31]. Thus,more work will be done in the field of the genetics of MMAF. In our following study ,more MMAF patients will be collected to carrying out whole-exome sequencing and

Transmission electron microscopy assessment.

Acknowledgments: The authors thank Bo Ma for assistance in preparing this manuscript

Disclosure of Interest: the authors declare no competing interests.

Ethics approval: This is a case study. The Ethics Committee has confirmed that no ethical approval is required.

Details of patient's consent: the patient's permission to publication was obtained.

Contribution to authorship: Yuhu Li and Yuqun Huang was responsible for the literature search . Data interpretation and writing of initial draf twas by Liuguang Zhang . Manuscript writing and

approval was by Liuguang Zhang.

Funding: the research was supported by no funding

References:

1. Amiri-Yekta A, Coutton C, Kherraf ZE, Karaouzene T, Le Tanno P, et al. Whole-exome sequencing of familial cases of multiple morphological abnormalities of the sperm flagella (MMAF) reveals new DNAH1 mutations. Hum Reprod 2016; 31: 2872–80.
2. Barthelemy C, Tharanne MJ, Lebos C, Lecomte P, Lansac J. Tail stump spermatozoa: morphogenesis of the defect. An ultrastructural study of sperm and testicular biopsy. Andrologia 1990; 22: 417–25.
3. Ben Khelifa M, Coutton C, Zouari R, Karaouzène T, Rendu J, et al. Mutations in DNAH1, which encodes an inner arm heavy chain dynein, lead to male infertility from multiple morphological abnormalities of the sperm flagella. Am J Hum Genet 2014; 94: 95–104.
4. Chemes HE, Brugo S, Zanchetti F, Carrere C, Lavieri JC. Dysplasia of the fibrous sheath: an ultrastructural defect of human spermatozoa associated with sperm Immotility and primary sterility. Fertil Steril. 1987;48:664–9.
5. Chunyu Liu, Mingrong Lv. Homozygous mutations in SPEF2 induce multiple morphological abnormalities of the sperm flagella and male infertility.Journalof Medical Genetics 57(1):jmedgenet-2019-10601
6. Chunyu Liu,Haruhiko Miyata. Bi-allelic DNAH8 Variants Lead to Multiple Morphological Abnormalities of the Sperm Flagella and Primary Male Infertility.The American Journal of Human Genetics Volume 107, Issue 2, 6 August 2020, Pages 330-341.
7. Clementine Wambergue, Raoudha Zouari.Patients with multiple morphological abnormalities of the sperm flagella due to DNAH1 mutations have a good prognosis following intracytoplasmic sperm injection◦ Human Reproduction, Vol.31, No.6 pp. 1164–1172, 2016
8. Coutton C, Vargas AS, Amiri-Yekta A, Kherraf ZE, Ben Mustapha SF, et al. Mutations in CFAP43 and CFAP44 cause male infertility and flagellum defects in Trypanosoma and human. Nat Commun 2018; 9: 686.
9. Da´vila Garza SA, Patrizio P. Reproductive outcomes in patients with male infertility because of Klinefelter's syndrome, Kartagener's syndrome, round-head sperm, dysplasia fifibrous sheath, and 'stump' tail sperm: an updated literature review. Curr Opin Obstet Gynecol 2013;25:229 – 246.
10. Deepa Talreja, Chirag Gupta.Oocyte Vitrification: A Comparative Analysis Between Fresh and Cryopreserved Oocytes in an Oocyte Donation Program. Fertility & Reproduction Vol.02,No.01,pp.9-13 (2020).
11. Dong FN, Amiri-Yekta A, Martinez G, Saut A, Tek J, et al. Absence of CFAP69 causes male infertility due to multiple morphological abnormalities of the flagella in human and mouse. Am J Hum Genet 2018; 102: 636–48.
12. Inaba K. Molecular basis of sperm flagellar axonemes: structural and evolutionary aspects. Ann N Y Acad Sci 2007; 1101: 506–26.
13. Lores P, Coutton C, El Khouri E, Stouvenel L, Givelet M, et al. Homozygous missense mutation L673P in adenylate kinase 7 (AK7) leads to primary male infertility and multiple morphological anomalies of the flagella but not to primary ciliary dyskinesia.Hum Mol Genet 2018; 27: 1196–211.

14. Mariem Ben Khelifa. Mutations in DNAH1, which Encodes an Inner Arm Heavy Chain Dynein, Lead to Male Infertility from Multiple Morphological Abnormalities of the Sperm Flagella.The American Journal of Human Genetics 94, 95–104, January 2, 2014
15. Mitchell V,Rives N,Albert M.Outcome of ICSI with ejaculated spermatozoa in a seried of men with distinct ultrastructural flagellar abnormalities.Hum Reprod 2006;21:2065-2074
16. Noone, P. G., Leigh, M. W., Sannuti, A., Minnix, S. L., Carson, J. L., Hazucha, M., et al. (2004). Primary ciliary dyskinesia: diagnostic and phenotypic features. Am. J. Respir. Crit. Care Med. 169, 459–467. doi: 10.1164/rccm.200303- 365OC
17. Nsota Mbango et al.Genetic causes of male infertility: snapshot on morphological abnormalities of the sperm flagellum .Basic and Clinical Andrology (2019) 29:2
18. R Azambuja, M Badalotti.Successful birth after injection of frozen human oocytes with frozen epididymal spermatozoa. Reproductive BioMedicine Online.Vol 11. No 4. 2005 449–451
19. Robert I. McLachlan, M.D.,Tomomoto Ishikawa, Ph.D.Normal live birth after testicular sperm extraction and intracytoplasmic sperm injection in variant primary ciliary dyskinesia with completely immotile sperm and structurally abnormal sperm tails.Fertility and Sterility Vol. 97, No. 2, February 2012 0015-0282
20. Sha YW, Xu X, Mei LB, Li P, Su ZY, et al. A homozygous CEP135 mutation is associated with multiple morphological abnormalities of the sperm flagella (MMAF).Gene 2017; 633: 48–53.
21. Sha, Y., Wei, X., Ding, L. et al. Biallelic mutations of CFAP74 may cause human primary ciliary dyskinesia and MMAF phenotype. J Hum Genet (2020). https://doi.org/10.1038/s10038-020-0790-2
22. Shen-Min Yang, Hai-Bo Li . Morphological characteristics and initial genetic study of multiple morphological anomalies of the flagella in China.Asian Journal of Andrology (2015) 17, 513–515
23. Shenmin Yang,Liang Gao.Successful intracytoplasmic sperm injection with testicular spermatozoa from a man with multiple morphological abnormalities of the sperm flagella: a case report. Assist Reprod Genet (2018) 35:247–250
24. Sil flflow, C. D. & Lefebvre, P. A. Assembly and motility of eukaryotic cilia and flflagella. Lessons from Chlamydomonas reinhardtii. Plant Physiol. 127, 1500–1507 (2001).
25. Stalf T, Sanchez R, Kohn FM, Schalles U, Kleinstein J, Hinz V, et al. Pregnancy and birth after intracytoplasmic sperm injection with spermatozoa from a patient with tail stump syndrome. Hum Reprod. 1995;10:2112–4.
26. Tao Huang,Yingying Yin. Absence of murine CFAP61 causes male infertility due to multiple morphological abnormalities of the flagella.Science Bulletin Volume 65, Issue 10, 30 May 2020, Pages 854-864.
27. Turner RM, Musse MP, Mandal A, Klotz K, Jayes FC, et al. Molecular genetic analysis of two human sperm fibrous sheath proteins, AKAP4 and AKAP3, in men with dysplasia of the fibrous sheath. J Androl 2001; 22: 302–15.
28. Wei-Li Wang, Chao-Feng Tu. Insight on multiple morphological abnormalities of sperm flagella in male infertility: what is new?.Asian Journal of Andrology (2020) 22, 236–245
29. XiaoliWang, YouhengWei. Tssk4 is essential for maintaining the structural integrity of sperm flagellum.Molecular Human Reproduction, Vol.21, No.2 pp. 136–145, 2015
30. Yanwei Sha,Xiaoli Wei,Lu Ding.DNAH17 is associated with asthenozoospermia and multiple morphological abnormalities of sperm flagella. Human Genetics Volume84, Issue3, May 2020, Pages 271-279
31. Ying Shen, Feng Zhang . Loss-of-function mutations in QRICH2 cause male infertility with multiple morphological abnormalities of the sperm flagella.Nature Communications.2019 Jan 25;10(1):433.doi: 10.1038/s41467-018-08182-x.

Hosted file

`title page.pdf` available at https://authorea.com/users/361195/articles/482696-outcome-of-vitrified-oocyte-injected-by-immotile-testicular-spermatozo-with-totally-multiple-morphological-abnormalities-of-the-sperm-flagella-a-case-report

Hosted file

figure.pdf available at https://authorea.com/users/361195/articles/482696-outcome-of-vitrified-oocyte-injected-by-immotile-testicular-spermatozo-with-totally-multiple-morphological-abnormalities-of-the-sperm-flagella-a-case-report

Hosted file

table.pdf available at https://authorea.com/users/361195/articles/482696-outcome-of-vitrified-oocyte-injected-by-immotile-testicular-spermatozo-with-totally-multiple-morphological-abnormalities-of-the-sperm-flagella-a-case-report