Medicina Reproductiva y Embriología Clínica (2020) 7, 98–106

Medicina Reproductiva y Embriología Clínica

www.elsevier.es/mrec

ASEBIR

ORIGINAL ARTICLE

Healthy baby born after ICSI with ejaculated immotile spermatozoa from a male Kartagener syndrome using Magnetic-Activated Cell Sorting (MACS) as a compliment of sperm preparation technique: A case report

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Received 11 October 2019; accepted 26 October 2020
Available online 20 November 2020

KEYWORDS
MACS;
Kartagener syndrome;
ICSI;
Immotile spermatozoa

Abstract Kartagener syndrome is an autosomal recessive genetic disorder associated with male infertility. Mutations in genes that encode a protein called dynein affect sperm motility.

A good option for these patients is to enhance the sperm selection for ICSI that could lead to improve the reproductive outcomes. For this purpose, different strategies have been used successfully in isolated cases.

In the present case report, we successfully applied MACS technique to a semen sample. This immunomagnetic method of sperm selection was aimed at reducing apoptosis manifestations, including DNA fragmentation, to optimize the outcomes of the ICSI procedure. By doing so, we increased the chances of selecting spermatozoa with superior quality and higher fertilization potential in the presence of total asthenozoospermia, even after incubation with pentoxifylline. Six of ten oocytes (60%) were appropriately fertilized. Two good-quality embryos were transferred on day 3 resulting in a pregnancy and a healthy baby born and one cavitated blastocyst was frozen on day 5.

MACS technique used as a compliment of sperm preparation technique may be a promising approach in routine IVF practice for men with Kartagener syndrome to improve reproductive outcomes optimizing the outcomes of the ICSI procedure.

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https://doi.org/10.1016/j.medre.2020.10.002

PALABRAS CLAVE
MACS;
Síndrome de Kartagener;
ICSI;
Espermatozoides inmóviles

Bebé sano nacido después de ICSI con espermatozoides inmóviles eyaculados de un síndrome de Kartagener masculino utilizando la clasificación de células magnéticas activadas como complemento de la técnica de preparación de esperma: un informe de un caso

Resumen El síndrome de Kartagener es un desorden genético autosómico recesivo asociado con la infertilidad masculina. Las mutaciones en los genes que codifican una proteína llamada dinaina afectan la motilidad del esperma.

Una buena opción para estos pacientes es mejorar la selección de espermatozoides para el ICSI que podría llevar a mejorar los resultados reproductivos. Para ello se han utilizado con éxito diferentes estrategias en casos aislados.

En el presente informe de casos, aplicamos con éxito la técnica MACS a una muestra de semen. Este método inmunomagnético de selección de espermatozoides tenía por objeto reducir las manifestaciones de apoptosis, incluida la fragmentación del ADN, para optimizar los resultados del procedimiento de ICSI. De este modo, aumentamos las posibilidades de seleccionar espermatozoides de calidad superior y con mayor potencial de fecundación en presencia de astenozoospermia total, incluso después de la incubación con pentoxifilina. Seis de diez ovocitos (60%) fueron fertilizados apropiadamente. Se transfirieron dos embriones de buena calidad el día 3, lo que dio lugar a un embarazo y al nacimiento de un bebé sano, y un blastocisto cavitado se congeló el día 5.

La técnica MACS utilizada como complemento de la técnica de preparación de esperma puede ser un enfoque prometedor en la práctica rutinaria de la FIV para hombres con síndrome de Kartagener para mejorar los resultados reproductivos optimizando los resultados del procedimiento ICSI.

Introduction

Kartagener syndrome (KS) is an autosomal recessive genetic disorder, affecting approximately one in every 15,000 live births. This congenital disease belongs to ciliary dyskinesia syndrome (CDS), which is characterized by the presence of several symptoms such as chronic sinusitis, bronchiectasis, *situs inversus,* and infertility, secondary to structural or functional defects in the action of the motile cilia (Brush et al., 2007; Fawcett et al., 1977).

In male patients, infertility is due to partial or total immobility of the spematozoon flagellum because of mutations on genes that control the synthesis of radial spokes or inner and outer dynein arms. These structures form temporary cross bridges between adjacent ciliary filaments and are believed to be responsible for generating movement in cilia and flagellum spermatozoa. Previous reports indicated mutations or deletions on these genes such as DNAI1, DNAI2, DNAH5, DNAH11, CCDC103, ARMC4, KTU/DNAAF2, LRRC50/DNAAF1, LRRC6, DYX1C1, ZMYND10, CCDC39, CCDC40, CCDC164, HYDIN, RSPH4A and RSPH1. Even a X-linked inheritance pattern was published in cases of CDS and KS (Raidt et al., 2014; Horani and Ferkol, 2018). Currently, 40 genes have been associated with disease, and >70% of patients tested have mutations in one of these genes.

Nowadays, intracytoplasmic sperm injection (ICSI) is considered to be the only reproductive strategy available in male patients affected by this syndrome (Nagy et al., 1998; Nijs et al., 1996). There have only been a few reports on successful fertilization and pregnancy using inmotile ejaculated sperm for ICSI where the main problem has been differentiating between live and dead sperm (Hattori et al., 2011; Ved et al., 1997; Soares et al., 2003).

In this line, magnetic-activated cell sorting (MACS), or magnetic separation by columns of annexin V, is applied in reproductive medicine in order to improve the sperm selection of assisted reproduction techniques. This technique is based on the ability of pre-apoptotic and dead spermatozoa with damaged plasma membranes and externalization of phosphatidylserine (PS) to bind to annexin V. This protein, when conjugated with super-paramagnetic microbeads, is retained along the separation column allowing non-apoptotic spermatozoa with intact membranes to go through the column when under the influence of a magnetic field (Paasch et al., 2007). Therefore, the selection of non-apoptotic spermatozoa can be used for enhancing assisted reproduction outcomes (Miltenyi et al., 1990; Grunewald et al., 2001, 2003; Paasch et al., 2004).

Over the past decade, several therapeutic approaches have been proposed to optimize the reproductive outcomes of infertile couples where the man is affected with primary ciliary dyskinesia (PCD). This study describes the case of a patient with Kartagener syndrome who achieved an ongoing pregnancy after MACS technique was applied to his ejaculated sperm. This immunomagnetic method is aimed at reducing apoptosis manifestations including DNA fragmentation, increasing the chance of selecting immotile sperm with superior quality.

Materials and methods

A couple with two years of primary infertility and without previous in vitro fertilization treatments (IVF) came to our fertility center.

The male, a 36-year-old man with no family history of the disease, was under suspicion of having Kartagener syndrome because of the presence of situs inversus (dextrocardia), recurrent upper respiratory tract infections and abnormal sperm analysis, characterized by total asthenozoospermia and teratozoospermia.

The morphology of sperm flagella was checked in another center under scanning electron microscopy, looking for alterations of dynein arms.

Subsequently, a genetic test was performed on blood samples, searching for the presence of mutations in the main genes associated with PCD.

After that, a karyotype and sperm-FISH (fluorescence in situ hybridization analysis on the sperm) were carried out. In the sperm-FISH analysis, chromosome anomalies were assessed in 2303 spermatozoa for chromosomes 18, X, and Y whereas 2018 spermatozoa were evaluated for chromosomes 13 and 21.

The basic semen analysis procedure was performed according to the World Health Organization (WHO) 2010 guidelines. Total asthenozoospermia was verified in spite of incubation with pentoxifilline (PF) (Sigma-Aldrich Quimica, Spain) 1.76 mM for 30 min (Yovich et al., 1990) and sperm viability was assessed by the eosin−nigrosin staining. Approximately equal volumes of semen and stain were mixed and incubated for 30 seconds (s) at room temperature. Then, a droplet was transferred to a slide where it was smeared. At least 200 spermatozoa were assessed at a magnification of 100× under oil immersion with an optical microscope (not phase contrast). White sperm (unstained) were alive and those that showed any pink or red color were classified as dead (WHO, 2010).

In this study, MACS technique was performed to eliminate apoptotic spermatozoa and obtain high quality sperm for the injection. The sperm vitality and DNA damage (spermatozoa with DNA fragmentation) was assessed before and after applying MACS.

Before beginning treatment, the couple received genetic counseling regarding the implementation of the indicated assisted reproduction techniques.

Controlled ovarian stimulation

The female, a 35-year-old woman, underwent controlled ovarian stimulation using a GnRH antagonist protocol (Orgalutran®, Merck, Spain) for pituitary suppression. Ovarian stimulation was carried out with a starting dose of 200 IU/day of recombinant follicle-stimulating hormone (rFSH) (Puregon®, Organon, Spain) for 11 days. A GnRH antagonist was added when one or more follicles of 13-mm mean diameter were visualized. Ovulation induction was performed with recombinant human chorionic gonadotropin (rhCG) 250 μg (Ovitrelle®, Merck, Spain) when at least 3 follicles reached a mean diameter of 17 mm, and oocyte retrieval was carried out 36 h later. The luteal phase was supported with 400 mg/day of natural micronized vaginal progesterone (Utrogestan®, Seid, Spain), starting 1 day after oocyte retrieval.

Sperm preparation for ART

After liquefaction at room temperature (25 °C), the ejaculated spermatozoa were prepared by centrifuging at 300xg and the pellet re-suspended with Glofert medium (Global® for fertilization, LifeGlobal, Belgium) in order to assess sperm concentration before magnetic separation. One aliquot of the sperm suspension served as a control and the other aliquot was subjected to MACS.

Sperm selection using the magnetic activated cell sorting (MACS) technique

For sperm magnetic selection, 5×10^6 sperm cells were incubated with 100 mL annexin V-conjugated MicroBeads (MACS® microbeads, Miltenyi Biotec, Spain) in the dark, at room temperature, and under continuous agitation for 30 minutes (min). The column containing iron globules was placed in the magnet (MiniMACS™, Miltenyi Biotec, Spain) and set up by rinsing 3 times with 500 ml of Binding Buffer (Miltenyi Biotec, Spain) before use. After the incubation period, 300 mL of Glofert were added to the sperm microbead suspension and, subsequently, the cell suspension was applied to the column. The annexin V negative fraction, containing nonapoptotic sperm, was eluted through the column and the annexin V positive fraction is retained in the separation column and collected (Said et al., 2005). Then, an aliquot of the annexin V negative fraction was washed and re-suspended in Glofert specific medium prior to the ICSI procedure.

Measurement of DNA fragmentation by TUNEL assay and flow cytometry analysis

DNA fragmentation was assessed before and after MACS technique using the TUNEL (terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate-biotin nick-end labeling) assay (Roche, Spain) as in the report (Liu et al., 2004), with minor modifications. Briefly, aliquots of the sperm samples were washed twice in phosphate-buffered saline (PBS) (Gibco® PBS, Life technologies, Spain) for 5 min, followed by centrifugation for collecting spermatozoa at $200 \times g$. The spermatozoa were then treated with a solution containing 0.1% Triton X-100 (Sigma-Aldrich Quimica, Spain) and 0.1% sodium citrate (Sigma-Aldrich Quimica, Spain) for 2 min on ice. A 30-ml TUNEL mixture consisting of terminal deoxynucleotidyl transferase (TdT) and fluorescein dUTP was added to the same volume of each sample. The samples were incubated for 60 min at 37 °C in a moist chamber in darkness, washed three times with PBS and then analyzed in a FACScan flow cytometer (Becton Dickinson, Spain). At least 10,000 cells were counted. The presence of green fluorescent signals was regarded as positive. For positive controls, spermatozoa were processed in the same way, except for a prior incubation with DNase I (1 mg/ml, D-4263; Sigma) for 10 min at room temperature to induce DNA fragmentation before the addition of the TUNEL mixture. For negative controls, the spermatozoa were similarly processed, but the TUNEL mixtures were added without the presence of TdT. The percentage of sperm with damaged DNA were calculated in a sperm aliquot before and after MACS technique. The established reference value of < 20% TUNEL-positive spermatozoa to be predictive for ICSI outcomes, was incorporated.

Oocyte retrieval and Intracytoplasmic Sperm Injection

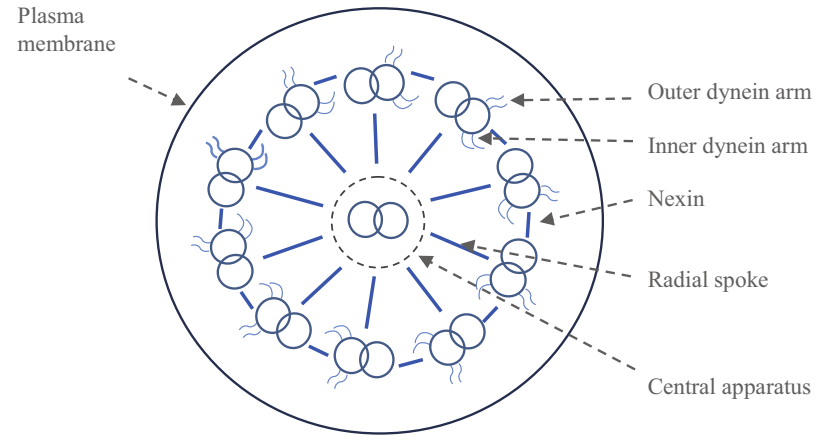

Figure 1 Schematic diagram that shows the microtubular structure. **Motile cilia 9+2** (normal ultrastructure).

All mature oocytes were micro-injected as described by Palermo et al. in 1992 with immotile spermatozoa after MACS. Sperm selection was also carried out according to the morphology and rigidity of the sperm flagellum. A rigid tail was considered a sign of non viability (Marques de Oliveira et al., 2004). After microinjection, oocytes were transferred into 50 μl drops of Glofert under mineral oil (LifeGlobal, Belgium) in a plastic dish and incubated at 37 °C under a 6.5% CO_2 atmosphere.

Fertilization was assessed 16–19 h after ICSI and only zygotes with the presence of two pronuclei and two polar bodies were considered normal. Only the successfully fertilized oocytes were cultured in dishes with drops of 50 μl of Global culture medium (Global®, LifeGlobal, Belgium).

Standard embryo evaluation was based on embryo development and morphological characteristics.

Results

Before the treatment, a genetic test was performed on blood samples, searching for the presence of mutations in the main genes associated with PCD, including DNAH5 and DNAI1, on chromosomes 5 and 9, respectively. Such mutations were not found on these regions. Up to now, many genetic PCD variants remain undiscovered, not all cases can be identified genetically. So that, negative genetic testing does not exclude diagnosis (Horani and Ferkol, 2018; Raidt et al., 2014).

On the other hand, karyotype and sperm-FISH analyses were normal for the analyzed chromosomes.

The morphology of sperm tails was checked under scanning electron microscopy to visualize the axoneme ultra-structure showing the absence of dynein arms (Fig. 1).

Urogenital examination showed no pathology and endocrine parameters were as follows: serum leves of follicle stimulating hormone (FSH) 3.91 mIU/mL, luteinizing hormone (LH) 4.14 mIU/mL and testosterone 378.2 ng/mL.

On the day of oocyte retrieval, the fresh semen evaluation showed a volume and concentration of 1.3 ml and 32×10^6/ml, respectively. Subsequently, 0.5 ml of the ejaculate (EJ) was washed and the pellet re-suspended in 0.2 ml Glofert. Total absence of motility in the sperm sample was confirmed, even after incubation with PF. The eosin-nigrosin test revealed 55% live spermatozoa.

Finally, 0.5 ml of the sperm sample concentrated at 0.2×10^6/ml was used for micro-injection after applying MACS technique. ICSI procedure was carried out with totally immotile spermatozoa avoiding those with completly rigid tail. Appliying TUNEL, the initial sperm fragmentation index in washed sperm was 23% (spermatozoa with fragmented DNA) and after MACS technique it was reduced to 11%. In the same way, the viability results in ejaculated spermatozoa before and after treatment were 55% and 60% respectively.

The female revealed no pathology and her basal endocrine assessment on the third day was normal: FSH 7.5 mIU/mL, LH 4.8 mIU/mL and estradiol 48 pg/mL.

After retrieval, 10 of the 14 oocytes were in metaphase II (71.4%). From sixteen to nineteen hours after ICSI, six of these 10 oocytes (60%) were appropriately fertilized. The presence of 3 pronuclei and 2 polar bodies was only observed in 1 zygote (10%), one of the zygotes (10%) with only 1 polar body and 0 pronuclei and 2 (20%) were degenerated. With regard to embryonic development on day 2, 4 of 6 embryos presented with 4 even cells with <10% fragmentation, and 1 had multinucleated blastomeres. Two less developed embryos (with 2 and 3 cells) were multinucleated and the second one had 25% fragmentation and was uneven. Embryo transfer was carried out on day 3. Two even 8- and 6-cell-embryos were transferred into the uterus. Three poor quality embryos with slower development were cultured until day 5. Of those, one cavitated blastocyst was frozen.

Thirteen days after the embryo transfer, the value of the woman's β-hCG blood test was 112.6 IU, and 10 days later the transvaginal ultrasonography showed the existence of one intrauterine gestational sac with heart activity at 7 weeks gestation. At this present moment, we know a healthy baby girl was born with normal pediatric developmental rate without situs inversus and recurrent respiratory infections. Nevertheless, there is a possibility that medical problems and other fertility problems will be diagnosed later. All patients must be informed about these risks and receive appropriate genetic counseling before the use of ICSI is considered.

Discussion

Previously, the main function of spermatozoa in fertilization and embryo development was reduced to being a carrier that transports DNA to the oocyte. But now, it is well known that the quality of both gametes play an important role that extends beyond the fertilization and embryo development directly influencing in the results (Said and Land, 2011; Sirard et al., 2006).

Literature states that total asthenozoospermia can arise from several mechanisms such as ultrastructural defects in the sperm tail related to genetic disorders, genital infections, antisperm antibodies, environmental pollutans, delayed epididimal transport and oxidative stress (Ortega et al., 2011). PCD is an autosomal recessive genetic disorder that impairs the action of cilia in the respiratory tract, fallopian tube and in the flagella of spermatozoa, leading to infertility problems in 50% of men with this pathology (Brush et al., 2007; Fawcett et al., 1977). Although there are different levels of sperm motility in these patients, most of them only present immotile spermatozoa in the ejaculate (Kay and Irvine, 2000; Von Zumbusch et al., 1998; Abu-Musa et al., 1999; Papadimas et al., 1997), as reflected in this case.

Even with the improvements in IVF techniques and the introduction of ICSI into routine laboratory procedures, total asthenozoospermia still exerts a negative effect on reproductive outcomes (Kahraman et al., 1996; Nagy et al., 1995).

In daily lab routin the embriologyst has inevitably to face the use of immotile spermatozoa. Therefore quick and easy laboratory methods to differentiate between dead and viable but immotile spermatozoa are required. For the purpose of sperm selection for ICSI, several studies have been published which investigated alternative techniques to implement in in vitro fertilization (IVF). Different strategies have been used in isolated cases with a wide range of results, such us the hypo-osmotic swelling test, pentoxifylline to enable movement, mechanical touch technique to test the flexibility of the sperm tail and laser-assisted immotile sperm selection, (Yildirim et al., 2009; Gerber et al., 2008; Ved et al., 1997; Marques de Oliveira et al., 2004; Hattori et al., 2011).

The first child born live after ICSI with immotile sperm from a male patient with Kartagener's syndrome was reported by Stalf et al. in 1995. Even though, we know that microinjection with these sperm provide poor outcomes, there have been several cases of asthenozoospermia related to dynein arm deficiency in which ICSI achieved healthy offspring (Table 1).

To the best of our knowledge, this is the second time that the use of MACS was reported in an attempt to improve ICSI procedures, particularly in males with Kartagener's syndrome. In the present report, selection of high-quality sperm including improvements in DNA integrity, resulted from the application of an advanced sperm selection technique (MACS) that represents another option to optimize the reproductive outcomes in males with an immotile sperm population. A previous research with sixteen patients showed that the combined use of density gradient centrifugation (DGC) and MACS is an efficient method for isolating viable spermatozoa, with lower level of DNA fragmentation and normal morphology (Zhang et al., 2018).

Several studies attempted to link apoptotic alterations in sperm (fragmentation) with conventional seminal parameters finding a significant negative correlation between the proportion of apoptotic cells and sperm motility, viability and normal morphology in ejaculated semen (Han-Ming et al., 2002).

Programmed cell death is an ongoing physiological phenomenon that has been documented to play a role in male infertility if deregulated (Avendaño et al., 2009; Boe-Hansen et al., 2006). Caspase activation, externalization of phosphatidylserine, alteration of mitochondrial membrane potential, and DNA fragmentation are markers of apoptosis found in ejaculated human spermatozoa. DNA damage consists of the presence of breaks in the sperm's genetic material, affecting the integrity and quality of the sperm sample. The greater the damage, the lower the integrity of the genetic material and the lower the likelihood of achieving an ongoing pregnancy. Recently, increased sperm DNA fragmentation was shown to be related to recurrent fertilization failure in a case of Kartagener syndrome (Nuñez et al., 2010). In human spermatozoa, typical apoptosis starts with the externalization of the PS phospholipid on the surface of the plasma membrane and finishes with DNA fragmentation (Glander and Schaller, 1999).

Taking this into account, the selection of non-apoptotic spermatozoa from the ejaculated may also prove to be beneficial in patients with this syndrome. This sperm selection technique, MACS, based on the high affinity of annexin V for phosphatidylserine, has been used as a non-invasive method in different fields in order to reduce the percentage of apoptotic cells (Said et al., 2006). The sperm selection process has become a good approach to better selection of good quality spermatozoa from ejaculated sperm to improve clinical outcomes in assisted reproductive treatments (Rawe et al., 2010). Many researches have proved the ability of MACS employing annexin V microbeads to enrich vital and motile sperm with inactivated apoptosis signaling and lower DNA fragmentation rate (Grunewald et al., 2001, 2006; Paasch et al., 2003, 2004). Moreover, Clinical studies and reports have showed improved ability to fertilize oocytes (Miltenyi et al., 1990; Paasch et al., 2004) and superior implantation and pregnancy rates, integrating this method as a unique molecular preparation technique that can compliment conventional sperm preparation protocols (Dirican et al., 2008; Rawe et al., 2010).

In the present case report, we successfully applied this sperm selection technique to a semen sample from a patient with Kartagener syndrome, aimed at reducing the percentage of damaged cells to optimize the outcomes of the ICSI procedure. By doing so, we increased the chances of selecting spermatozoa with superior quality and higher fertilization potential in the presence of total asthenozoospermia. The percentage of spermatozoa stained with eosin-nigrosin was slightly lower after MACS. This observation may be explained by the fact that the spermatozoa with apoptotic markers, susceptible to annexin V binding at an early stage of apoptosis, are not always related to dead sperm while the staining is being carried out (Kotwicka et al., 2011).

Table 1 Reproductive results of ICSI using totally immotile ejaculated sperm (a brief chronological summary).

Sperm origin	Male age	Female age	Sperm sample	Sperm selection method	Fertilization rate (%)	Cleavage blastocyst embryos transferred	Livebirth clinical pregnancy	References
EJ	40	38	40×10^6/mL 13×10^6/mL	ICSI ICSI	50% (1/2) 40% (2/5)	Transferred embryo on day 2 (4 cells) Transferred embryos on day 2 (4 and 2 cells)	No pregnancy No pregnancy	Papadimas et al., 1997
EJ	Median male age 36 years (four couple)	Median female age 31.3 years (four couple)	Sperm concentration (range of $4–45 \times 10^6$/mL)	ICSI	The average fertilization rate 53% (19/36)	2 cases (2 embryos transferred) and in the other 2 (4 embryos transferred)	One miscarriage and Live birth of twins	Barros et al., 1997
EJ	34 36	32 –	Case 1: 75×10^6/mL Case 2: 210×10^6/mL	ICSI	Case 1: 66.6% (4/6) Case 2: 50% (3/6)	Case 1: 2 preimplantation embryos at the 4-cell stage were transferred Case 2: 3 preimplantation embryos at the 4–6 cell stage were transferred	Live birth of healthy twins (case 1) and a singleton (case2)	von Zumbusch et al.,1998
EJ	36	30	58×10^6/mL	ICSI	0% (0/4)	–	No fertilization and pregnancy	Abu-Musa et al., 1999

Table 1 (*Continued*)

Sperm origin	Male age	Female age	Sperm sample	Sperm selection method	Fertilization rate (%)	Cleavage blastocyst embryos transferred	Livebirth clinical pregnancy	References
EJ vs TESE	41 33	35 28	Case 1: severe oligozoospermia Case 2: normal sperm count and morphology 63% viability with HOS test. Both total immotility	Case 1. HOS test and ICSI Case 1. TESE, HOS test and ICSI Case 2: ICSI after HOS test with half sperm injected from EJ and other half TESE.	Case 1. 0% (0/7) Case 1. 75% (6/8) Case2. 44% (4/9) 56% (5/9) respectively	Case 1. – Case 1. 2 embryos on day 3. 3 embryos were cryopreserved. Case 2. One day 3 embryo (from testicular sperm) and 6 embryos were cryopreserved.	Case1. 1st attempt: No fertilization Case1. 2nd attempt: Live birth of twins Case3: pregnancy (embryo derived from TESE)	Westlander et al., 2003
EJ	38	–	43×10^6/mL,	HOS test and ICSI	50% (5/10)	4 transferred embryos (4 cell and 2 cell)	Live birth of twins	Geber et al., 2008
EJ	36	28	32×10^6/mL 40% viability by eosin-nigrosin test.	ICSI following HOS test	83.3% (5/6)	5 blastocyst (100%) and 2 blastocysts were transferred and 3 blastocysts were cryopreserved	Live birth of twins	Kordus et al., 2008
EJ	31	30	0.9×10^6/mL, 54% viability by eosin test. Slight sperm motility with pentoxifiline	pentoxifylline activated sperm pick by ICSI	58.3% (7/12)	Blastocyst formation rate was 57.1% (4/7)	Live birth of a singleton baby	Hattori et al., 2011
EJ	36	33	5×10^6/mL	Laser assisted viability assay (LAVA) shooting the sperm tails during sperm retrieval for ICSI	45.5% (10/22)	4 blastocyst Blastocyst rate 40% (4/10) DET	Live birth of three babies	Ozkavukcu et al., 2018

Although, this particular case has shown clear benefits appliying this technique in a male with this pathology, further comparative studies with a larger number of patients would be recommended to confirm these findings.

Taking this into consideration and based on our good results, having achieved an almost average fertilization rate, we conclude that MACS may be a promising approach in routine IVF practice for men with Kartagener syndrome, improving reproductive outcomes. This immunomagnetic method is aimed at reducing apoptosis manifestations including DNA fragmentation, increasing the chance of selecting immotile sperm with superior quality.

Conflict of interests

The authors declare that they have no conflict of interest.

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