CASE REPORT

Pregnancy resulting from IMSI after testicular biopsy in a patient with obstructive azoospermia

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Abstract

In this article, we report a case of pregnancy obtained in an infertile couple diagnosed with severe male factor infertility. The couple attended for fertility examination reporting a history of 10 years of infertility. The cause of infertility was obstructive azoospermia. The treatment consists of in vitro fertilization (IVF). The ovarian stimulation of female patient was done with antagonist protocol and after ovarian puncture was obtained nine oocytes. The urologist performed testicular sperm extraction (TESE). There were selected nine sperm cells by intracytoplasmic morphologically selected sperm injection (IMSI). For this purpose, we used an inverted microscope with high magnification equipped with ×60 air objectives with modulation contrast illumination. After intracytoplasmic sperm injection (ICSI) of sperm into the oocytes there were obtained six normal embryos from which three embryos were transferred into the uterus. A singleton pregnancy was achieved which was completed with birth of a healthy baby in time. This successful outcome shows that use of IMSI and ICSI procedures are really useful in selection of best spermatozoa obtained by TESE in treatment of obstructive azoospermia.

Keywords: azoospermia, in vitro fertilization, oocytes, testicular sperm extraction.

Introduction

Azoospermia, defined as complete absence of sperm from the ejaculation, is present in less than 1% of all men and in 10 to 15% of infertile men. Although there are many causes of azoospermia, obstruction of the ductal system is responsible for approximately 40% of cases [1].

Men with obstructive azoospermia may produce a pregnancy either by:

• surgical correction of the obstruction, which may produce pregnancy by intercourse and obviate the need for assisted reproductive technology;

• retrieval of sperm from the male’s reproductive system for in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI).

Testicular sperm retrieval and its use in the treatment of infertile couples was performed for the first time in 1995 [2].

Sperm can be obtained either aspirated by puncture from the epididymis (PESA – percutaneous epididymal sperm aspiration) or testicle (TESA – testicular sperm aspiration), or extracted from the testicle by biopsy [3]. Micro-dissection sperm retrieval (TESE – testicular sperm extraction) performed with an operative microscope is generally considered to be the best method with higher spermatozoa retrieval rates and minimal tissue loss in azoospermic patients [4]. At present, there are no options for men with azoospermia and failed TESE to have biological children [5].

Since sperm cells retrieval by these invasive methods comes from subjects with important sperm pathology, the obtained sperm cells are extremely few and show major changes in motility and morphology.

Oocyte penetration is possible with intracytoplasmic sperm injection (ICSI) techniques.

The head of the sperm that contains the cell nucleus and the entire genetic information is injected inside the oocyte using micropipettes handled by a special micromanipulation system attached to an inverted microscope [6].

However, the low magnification used in routine ICSI microscopy (×200, ×400) results in limitations in identifying sperm organelle malformations, particularly vacuoles in the sperm head, where major defects, such as abnormal sperm head size proportions and midpiece abnormalities were observed [7].

For a better morphology assessment of mobile spermatozoa, in the last years there has been imagined a new technique for selecting sperm. Bartoov et al. have developed a new method known as motile sperm organelle morphology examination (MSOME), which evaluates the real-time movement of sperm [8, 9]. They made it possible to obtain a sperm with a normal nucleus by combining MSOME and micromanipulation technology. This process is known as the modified IVF process or intracytoplasmic
morphologically selected sperm injection (IMSI) because only one moving sperm is selected when performing ICSI using MSOME [7]. IMSI uses a high microscope magnification ×600 and resolution is associated with better embryo quality and higher rates of implantation and pregnancy [10]. IMSI procedure is a good option for couples with a first unsuccessful ICSI cycle [11].

In this article, we present a successful case of TESE followed by IMSI and ICSI in a couple of male infertility due of obstructive azoospermia. The procedure was performed in 2015 and was completed with the birth of a healthy baby in time.

Case presentation

The couple attended for fertility examination reporting a history of 10 years of infertility. Age of the patient was 31 years old. He was diagnosed with azoospermia by performing spermograms in four different laboratories. No other pathology was present in the male. Urological examination was normal.

Obstructive azoospermia was diagnosed by evaluating hormonal status of the patient that revealed normal values: FSH (follicle-stimulating hormone) 4.26 IU/L, LH (luteinizing hormone) 6.32 IU/L, testosterone 12.52 ng/mL, TSH (thyroid-stimulating hormone) 2.5 IU/L and prolactin 668 mIU/L.

Sperm and urethral cultures were normal. The sperm antibodies were absent to both partners. The genetic examination showed normal karyotype with absence of micro-deletions on the Y chromosome or mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene.

The woman was 29 years old whose hormonal status indicated an increased ovarian reserve on day 2 of the cycle: FSH 6.5 IU/L, LH 13.3 IU/L, E2 (estradiol) 53 ng/mL, AMH (anti-Müllerian hormone) 4.6 ng/mL. There were no clinical features of polycystic ovarian syndrome.

An informed consent was obtained for treatment procedure.

The woman received controlled ovarian stimulation that lasted 11 days. The ovarian stimulation was done with the antagonist protocol and using 20 ampoules of 75 IU of FSH + LH (Menopur, Ferring) and six ampoules of Ganirelix (Orgalutran, MSD).

Ovulation was triggered with 250 μg rHCG (recombinant human chorionic gonadotropin – Ovitrelle, Serono).

They were obtained nine oocytes, which had undergone in vitro fertilization procedure.

The urologist performed testicular biopsy by classic technique under intravenous anesthesia, making unilateral hemiscrotomy with minimal incision and testicular tissue sampling. The test was carried out by micro-dissection of the testis.

A piece of testicular tissue was investigated for histopathological changes (Figure 1). Harvested tissue was fixed in 10% buffered formalin solution and embedded in paraffin. Serial sections were made with a thickness of 3 μm. These were stained with Hematoxylin–Eosin (HE) and examined at the optical microscope at ×100 magnification. The pathologist observed discreet interstitial fibrosis with mild thickening of the seminiferous tubules own tunic. It also observed an appreciable reduction in the number of germ cells in some areas disorganized and degenerate, spermatogonia cells, primary spermatocytes, very rare elongated spermatids and sperms.

Another piece of testicular tissue was analyzed by the embryologist. Perioperative microscopically examination of a wet preparation of the shredded specimens was performed at ×400 ICSI magnification according to a technique described by Jow et al. [12]. The embryologist noted the existence of a very small number of morphologically normal spermatozoa that had minimal motility. There was observed an elevated proportion of immature germ cells belonging to immature germ lines: spermatogonia, spermatocyte and spermatid. Mature forms showed abnormal morphology of the head and neck of sperm. The embryologist excluded from ICSI spermatozoa exhibiting severe head defects, which were clearly seen at this magnification: pin, amorphous, tapered, round, and multi-nucleated head (Figure 2).

Figure 1 – Testicular tissue obtained by TESE. Rare seminiferous tubules, which present advanced sclerosis correlated with appreciable reduction of germ cells (HE staining, ×100).

Figure 2 – Testicular tissue obtained by TESE. We notice the presence of sperm cells from germ-line and rare mature forms represented by sperm with atypical forms (freshly prepared, ×400).

Few mature spermatozoa were selected by IMSI under high magnification using an inverted microscope Nikon Eclipse Ti-U equipped with modular contrast Hoffman and micromanipulation system Narishige. This system is adapted for the observation and manipulation of living cells; it is equipped with a transparent thermoplate at 37°C (Tokai Hit). The microscope incorporated a ×60 air objectives with modulation contrast illumination (RI IMSI,
The introduced intracytoplasmic sperm injection procedures into the techniques of assisted reproduction marked an important milestone in the development of the field [14]. With this procedure, patients whose semen samples were insufficient for intrauterine insemination or in vitro fertilization techniques could achieve pregnancy using micromanipulation techniques [15]. Spermatozoa morphology is the best selection criteria for intracytoplasmic injection. Several studies demonstrated a positive correlation between optimal sperm morphology evaluation and ICSI outcomes improvement. De Vos et al. evaluated the impact of individual sperm morphology on ICSI outcome: fertilization, embryo development and implantation rate [16]. This study was performed by using an inverted light microscope and sperm cells were classified as normal in accordance to Kruger et al. criteria [17]. The authors demonstrated that the injection of abnormal spermatozoa was associated to a significantly lower fertilization, implantation and pregnancy rate when compared to ICSI cycles performed by injecting spermatozoa with apparently normal morphology. The low magnification and resolution of the microscope used for the morphology assessment represented the main limitation of this study. However, due to relatively low power magnification (<200×400), ICSI technique has its limits in terms of changes in intracellular characterization [18].

IMSI technique currently is the only real-time microscopy procedure who does not require fixation and who can select viable sperm with minimal ultrastructural defects [7]. Indeed, most of the enzymatic or genetic tests currently available cannot be performed on viable, unfixed,
spermatozoa. The detection of large nuclear vacuoles at high magnification could be related to DNA fragmentation and denaturation. It is widely accepted that DNA integrity plays an important role in the fertilization process and in the development and implantation of embryos [19, 20]. Furthermore, Vanderzwalmen et al. reported a negative influence of spermatozoa with large nuclear vacuoles in the head on the capability of embryos to develop to the blastocyst stage [21]. Antinori et al. pointed out the need to increase the efficiency of micro-insemination techniques especially in those countries, such as Italy, where the law limits the number of fertilizable oocytes [22]. Several studies demonstrated that IMSI provided positive results in couples with severe male factor infertility or repeated ICSI failures [8, 23, 24]. In all these studies, the clinical pregnancy rate was noticeably improved in the IMSI group. Moreover, the IMSI group result was associated with a lower abortion rate [10, 17].

IMSI technique for selecting sperm morphology has proved useful in choosing cells with maximum potential to give rise to viable and good quality embryos, considering that their harvest was performed by micro-dissection of testicular biopsy tissue [9].

The number, mobility, maturity and sperm morphology was negatively influenced by intrinsic testicular pathology and invasive microsurgery technique followed by micro-dissection [8].

Obviously, every single good-quality oocyte deserves the best spermatozoa available in the sperm sample to be used for microinjection, in order to obtain the highest probability of developing a high quality embryo that implants. It needs to be defined what is absolutely needed in terms of normalcy presenting with no single vacuole in the sperm head on the one hand and what is at least as good or good enough to be used as second-best spermatozoa on the other hand without compromising oocyte fertilization, embryo development and implantation potential.

Using testis biopsy sperm for azoospermia may gain the same high pregnancy rate as the normal ejaculation sperm, but efficiency of spermatid application is doubted because low fertilization and pregnancy rates influence seriously its success. As cell-cloning technology develops, other options for treatment of azoospermic men include use of secondary spermatocytes, sperm cell cloning, and artificial sperm production by inducing stem cells and adult somatic cells into sperm cells. Although these technologies still remain at animal research stage and demonstrate a low efficiency, we believe that application of these novel techniques will greatly improve chance for infertile couples (particular azoospermic men) to conceive their own biological offspring in the next decade.

IMSI is a promising new technique that may help to improve laboratory and clinical performance in assisted reproduction. In cases of azoospermia, the combination of ICSI with getting sperm through TESE represents an edge therapy of male infertility.

In our case, the embryos’ quality obtained was demonstrated by the birth of a healthy term baby.

After our knowledge, this is the first case in Romania of pregnancy obtained through in vitro fertilization technique IMSI with ICSI on the sperm achieved by testicular biopsy.

Conclusions

The ICSI method involves a magnification of ×200–×400 enabling a relatively coarse selection. The embryologist excludes from ICSI spermatozoa exhibiting severe morphological defects, which are clearly seen at this magnification. The selection with IMSI is performed under ultra-magnification (×6000) with possibility to detect the ultrastructure of the head, midpiece and its organelles. This new technique has a theoretical potential to improve reproductive outcomes among couples undergoing assisted reproduction techniques. In our case, IMSI help us to select the best spermatozoa in condition in which the number, mobility, maturity and morphology of these germinal cells were negatively influenced by intrinsic testicular pathology and invasive microsurgery technique followed by micro-dissection. In light of improved pregnancy rate and delivery rate, we tend to recommend promoting the IMSI method for couples with obstructive azoospermic male infertility. Further trials are necessary to improve the evidence quality before recommending IMSI in clinical practice.

Conflict of interests

The authors declare that they have no conflict of interests.

Consent

Written informed consent was obtained from the couple of patients for publication of this Case Report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of Rom J Morphol Embryol.

References

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