http://www.cjmb.org

Open Access Case Report

Crescent Journal of Medical and Biological Sciences
Vol. 6, No. 1, January 2019, 129–131
eISSN 2148-9696

# A Novel Therapy for the Treatment of Malefactor Infertility Due to Non-obstructive Azoospermia: A Case Report

**Mohammed Iqbal Cassim[1], Tasneem Mohamed[1*]**

## Abstract

A case report on a novel treatment protocol using autologous stem cells, derived from adipose tissue, for the treatment of non-obstructive azoospermia. In this case report, the male partner after undergoing such treatment had restored spermiogenesis and the couple underwent in vitro fertilization (IVF) therapy. Fertilization was successful and good quality embryos were produced.

**Keywords:** Non-obstructive azoospermia, Infertility, Stem cell therapy

## Introduction

The advent of assisted reproductive technology has afforded many previously "infertile" couples the gift to produce offspring. Through various techniques like ovulation induction, artificial insemination, in vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI), many pathologies responsible for infertility have been overcome. However, male factor infertility due to primary non-obstructive azoospermia remains a challenge for the couple, as well as for the attending infertility specialist.

Azoospermia is the total absence of sperm in the ejaculate. It may be obstructive or non-obstructive. In the obstructive type, sperm may be found in the testes but not in the ejaculate due to either congenital or acquired obstruction of the ejaculatory duct. Non-obstructive azoospermia, on the other hand, is due to an inability of the testes to produce sperm either due to the fact that there is inadequate central stimulation of the testes or an abnormality within the testes whereby the testis is not capable of spermiogenesis.

To date, various treatments using stem cells are being explored with variable success to treat azoospermia and enable the infertile male to father a biological child. We report on a novel treatment protocol using adult adipose-derived stem cells to restore spermatogenesis leading to the in vitro production of good grade embryos.

## Case Report

This report is about a couple with primary infertility attending a fertility center in Johannesburg. The male, aged 36 was diagnosed with idiopathic non-obstructive azoospermia, confirmed on multiple semen analysis and at least two testicular biopsies, both with histological Johnsen score of 5 (1). Notably, the second testes biopsy was preceded by a course of gonadotrophin therapy with no change in the Johnsen score. His most recent serum FSH and LH levels were 7.7 U/L and 8.9 U/L respectively and his free testosterone level was 298.1 pmol/L. Chromosomal analysis revealed a normal male karyotype, and investigations for cystic fibrosis and bilharzia were normal. The option of stem cell therapy was discussed with the couple and they agreed to undergo 4 sessions of stem cell therapy using autologous mesenchymal stem cells harvested from adipose tissue. (See attached JirehStem protocol – Supplementary file 1). The adipose tissue used was from the subcutaneous layer of the abdominal wall and was aspirated via a simple liposuction procedure using a suction apparatus. The stem cells were then harvested from the adipose tissue and activated using the Adistem processing unit (see Figure 1). The initial 3 sessions consisted of intravenous infusions (IVIs) of stem cells at fortnightly intervals.

The fourth session was done one month after the last IVI therapy and consisted of bilateral testicular stem cell infiltration/injection. Stem cells were prepared as per technique of the stem cell specialist. 0.5 mL of the preparation was injected into the right testis via several insertions and then into the left testis via 1 insertion with the needle being moved circumferentially. An ultrasound of the testes was done after the procedure to ensure good depth of stem cell injection. Gonadotrophin therapy was started 2 days later with daily sc injection of 75 IU of highly purified hMG (Fostimon, IBSA) and 75 IU of

**Received** 14 December 2017, **Accepted** 8 July 2017, **Available online** 3 August 2018

[1] Bio ART Fertility Center, Rosebank, South Africa.
***Corresponding Author:** Tasneem Mohamed, Tel: +27 11 484 5119, Email: tm.mohamed@yahoo.com

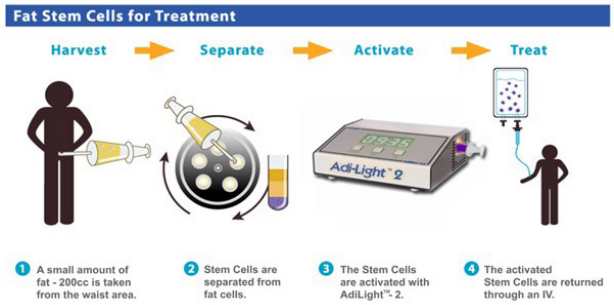


**Figure 1.** Adipose Stem Cells Processing (Adistem).

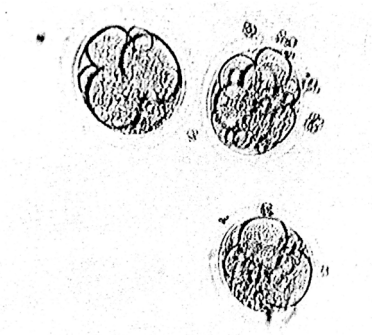

**Figure 2.** Microscopic Image of Cultured Embryos on the Day of Transfer.

recombinant FSH (Gonal F, Merck/Serono). A testicular biopsy was scheduled for 3 months later.

Patient compliance was good and no adverse side effects were reported. However, the patient only came for an evaluation 4 months later. A simple semen analysis was done and the presence of sperm was noted in the ejaculate. The sample was cryopreserved. The couple then went on to have an IVF cycle using the step-up protocol of the fertility center. Three good grade embryos were successfully cultured and transcervically transferred into the endometrial cavity.

Unfortunately, the embryos failed to implant and no pregnancy occurred.

## Discussion

Stem cells are undifferentiated cells found in all stages of life: the embryo, fetus and adult. They have a unique characteristic known as potency, which means they can give rise to differentiated cells of various cell lineages. They have regeneration and self-renewal capabilities, thus ensuring continuity of the cell line and repair of the organ following injury. Stem cells are "the building blocks of tissues and organs" (2).

Cells found in the early stages of embryo development are the most undifferentiated cells and are termed totipotent or omnipotent. Pluripotent stem cells are able to give rise to cells from all 3 germ layers, so essentially into any tissue or organ. Mesenchymal cells are multipotent stem cells. They are able to differentiate into mesodermal tissues such as adipose tissue, bone, cartilage and muscle (2).

Stem cells can be obtained from either embryos or adults. Embryonal stem cells have the advantage of being pluripotent but there are many ethical concerns surrounding the manner in which they are obtained (2). It has become possible to derive "induced pluripotent stem cells" from any tissue in the body using "a cocktail of reprogramming factors" (3). This process is transdifferentiation, whereby a cell from one germ cell line is able to differentiate into cells of another germ cell line (4). The ability to derive male germ cells from induced pluripotent stem cells is groundbreaking in the field of reproductive medicine and has increased the number of treatment options for males with medically induced (iatrogenic) azoospermia as well as those with idiopathic non-obstructive azoospermia (3).

For some males with non-obstructive azoospermia, sperm may be extracted through a testicular aspiration or biopsy and intracytoplasmic sperm injection may be sufficient to achieve a pregnancy (5), but for others, as is the case with our patient, testicular biopsy reveals no viable sperm. For patients with medically induced azoospermia like those undergoing radiation and chemotherapy for the treatment of cancer, adult males can be offered the option to cryopreserve semen prior to the initiation of therapy. However, unfortunately, this is not an option for pre-pubertal males (3). A study by Kurkure et al has shown that very small embryonic-like stem cells (VSELs) may be identified on testicular biopsy of azoospermic adults who have survived childhood cancer. As has been shown in studies on mice, these persisting VSELs have the potential to restore spermatogenesis (6). Another study by Jahnukainen et al investigated the cryopreservation of immature primate testicular tissue and the potential of spermatogonial stem cells. They have shown that this may be a possible tool for the preservation of future fertility in prepubertal boys (7).

Male germ cells have been successfully derived though transdifferentiation from bone marrow stem cells (8). These cells have also shown potential to recover spermatogenesis when transplanted into the seminiferous tubules of chemotherapy-induced azoospermic rat and hamster models (9,10).

More recently, mesenchymal stem cells in addition to bone marrow have been harvested from adipose tissue. This method has been found to be less invasive, less expensive and to contain more stem cells than aspirated bone marrow (11). These adipose tissue-derived mesenchymal cells have been shown to have the ability to repair the germinal cells of seminiferous tubules of chemotherapy-induced azoospermic rats (11). Other studies in induced azoospermic rats have further gone on to show successful spermatogenesis after stem cell treatment and also continuous generations in offspring (12).

Even though a pregnancy was not achieved in the presented case, this is a remarkable breakthrough for reproductive medicine. To the best of our knowledge, this is the first case to be published whereby sperm was noted in the ejaculate of a human subject with non-obstructive azoospermia following adipose derived mesenchymal stem cell therapy. The successful development of viable embryos in vivo is also extremely exciting and promising for future attempts at the successful achievement of pregnancy and live births.

## Acknowledgments

I would like to thank:

- The couple described in this report for allowing me to write up this case;
- The staff at BioART Fertility Center for assistance in providing the relevant technical information and images in order to compile this report; and
- A special thank to JirehStem for providing me with the stem cell protocol and the accompanying Adistem image.

## Conflict of Interests

Authors have no conflict of interests.

## Ethical Issues

The couple gave written consent to the treatment with stem cells and also provided consent for the publication of this report.

## Financial Support

Nil.

## Supplementary Materials

Supplementary file 1. Protocol for Small Fat Kit Procedure.

## References

1. Johnsen SG. Testicular biopsy score count--a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones. 1970;1(1):2-25. doi:10.1159/000178170
2. Volarevic V, Bojic S, Nurkovic J, et al. Stem cells as new agents for the treatment of infertility: current and future perspectives and challenges. Biomed Res Int. 2014;2014:507234. doi:10.1155/2014/507234
3. Gassei K, Orwig KE. Experimental methods to preserve male fertility and treat male factor infertility. Fertil Steril. 2016;105(2):256-266. doi:10.1016/j.fertnstert.2015.12.020
4. Barzilay R, Melamed E, Offen D. Introducing transcription factors to multipotent mesenchymal stem cells: making transdifferentiation possible. Stem Cells. 2009;27(10):2509-2515. doi:10.1002/stem.172
5. Ravizzini P, Carizza C, Abdelmassih V, Abdelmassih S, Azevedo M, Abdelmassih R. Microdissection testicular sperm extraction and IVF-ICSI outcome in nonobstructive azoospermia. Andrologia. 2008;40(4):219-226. doi:10.1111/j.1439-0272.2008.00846.x
6. Kurkure P, Prasad M, Dhamankar V, Bakshi G. Very small embryonic-like stem cells (VSELs) detected in azoospermic testicular biopsies of adult survivors of childhood cancer. Reprod Biol Endocrinol. 2015;13:122. doi:10.1186/s12958-015-0121-1
7. Jahnukainen K, Ehmcke J, Hergenrother SD, Schlatt S. Effect of cold storage and cryopreservation of immature non-human primate testicular tissue on spermatogonial stem cell potential in xenografts. Hum Reprod. 2007;22(4):1060-1067. doi:10.1093/humrep/del471
8. Nayernia K, Lee JH, Drusenheimer N, et al. Derivation of male germ cells from bone marrow stem cells. Lab Invest. 2006;86(7):654-663. doi:10.1038/labinvest.3700429
9. Zhang D, Liu X, Peng J, et al. Potential spermatogenesis recovery with bone marrow mesenchymal stem cells in an azoospermic rat model. Int J Mol Sci. 2014;15(8):13151-13165. doi:10.3390/ijms150813151
10. Tamadon A, Mehrabani D, Rahmanifar F, et al. Induction of Spermatogenesis by Bone Marrow-derived Mesenchymal Stem Cells in Busulfan-induced Azoospermia in Hamster. Int J Stem Cells. 2015;8(2):134-145. doi:10.15283/ijsc.2015.8.2.134
11. Mehrabani D, Hassanshahi MA, Tamadon A, et al. Adipose tissue-derived mesenchymal stem cells repair germinal cells of seminiferous tubules of busulfan-induced azoospermic rats. J Hum Reprod Sci. 2015;8(2):103-110. doi:10.4103/0974-1208.158618
12. Cakici C, Buyrukcu B, Duruksu G, et al. Recovery of fertility in azoospermia rats after injection of adipose-tissue-derived mesenchymal stem cells: the sperm generation. Biomed Res Int. 2013;2013:529589. doi:10.1155/2013/529589