

NJSC "Astana Medical University"

ANNOTATION

dissertation work of Zhankina Rano Amirkhanovna on the topic: "Possibilities of autotransplantation of mesenchymal stem cells in the treatment of male infertility", submitted for the degree of Doctor of Philosophy (PhD) in the specialty 8D10102 - "Medicine"

Relevance.

Infertility in marital marriage is the most important medical and social problem, drawing the attention of many researchers to the problem of reproductive health of the population [Glybochko P.V., Alyaev Yu.G., Chaly M.E. et al. 2013]. Infertility affects approximately 15% of couples of reproductive age [Vij S.C., Sabanegh E.Jr., Agarwal A., 2018]. Most of the male causes of infertility in a married couple range from 18.8 to 39% [Winters B.R., Walsh T.J., 2014]. Among patients with infertility, approximately 10-15% have azoospermia [Cocuzza M., Alvarenga C., Pagani R., 2013].

Azoospermia is perceived as the absence of spermatozoa in the spermogram and is approximately detected in 1% of men and in 10-15% of patients with infertility [Jarow J.P., Espeland M.A., Lipshultz L.I.]. Non-obstructive azoospermia is possibly the cause of male infertility, which is indispensable without drug therapy [Kumar R., 2013]. Married couples diagnosed with non-obstructive azoospermia; the only and last chance to have children is testicular sperm extraction with intracytoplasmic sperm injection (TESE-ICSI) [Hendriks S., Dancet E.A., Meissner A., 2014]. However, this type of intervention has a low success rate, since the finding and detection of spermatozoa in the first cycle of testicular sperm extraction is only 56% [Dabaja A.A., Schlegel P.N., 2013]. Married couples with non-obstructive azoospermia do not have the opportunity to have their own children clinically and have the opportunity to either adopt or use donor sperm [Chiba K., Enatsu N., 2016; Palermo G., Joris H., Devroey P.].

The advantages of ART as intracytoplasmic sperm injection [Palermo G., Joris H., Devroey P.], IVF have changed the approach to managing such patients with non-obstructive azoospermia.

Results in biotechnology have increased the ability to treat men with non-obstructive azoospermia [Chiba K., Enatsu N., 2016].

Mesenchymal stem cell therapy has been recognized as a new treatment option for non-obstructive azoospermia [Cyranoski D., 2013]. Lue et al. (2007) showed that bone marrow-derived mesenchymal stem cells transplanted into the testicles of animals, in particular rats with busulfan-induced azoospermia, caused differentiation into Sertoli and Leydig cells [Cyranoski D., 2013].

In 2016, a study performed in Jordan reported that scientists injected CD34+/CD133+ cells intratesticularly. After transplantation, the patients were followed up for 5 years. These patients did not have any complications. In 9 out of 27 patients, changes were found during histological examination [Al Zoubi A.M., 2014].

Thus, based on these premises, we came up with the idea of developing a strategy for the treatment of non-obstructive azoospermia using mesenchymal stem cells.

From a practical point of view, the results of the work can serve as the basis for the application of a new cellular therapeutic approach for the treatment of non-obstructive azoospermia using mesenchymal stem cells.

The purpose of the study: To evaluate the efficacy and safety of using autologous bone marrow mesenchymal stem cells in the treatment of non-obstructive azoospermia.

Object of study:

The present study was based on 25 patients diagnosed with non-obstructive azoospermia. Of the 25 patients, only 19 patients, according to the inclusion criteria, entered the pilot study between the ages of 24 and 48 years. The recruitment of patients was carried out sequentially in the process of outpatient admission. These are the patients who repeatedly applied to urologists, andrologists, reproductologists without a positive result. Clinical examination of patients was carried out at the bases: clinic "ECOMED plus", on the basis of the Center for Cellular Technologies, Transplantation and Management of the Institute of Fundamental and Applied Medicine JSC "National Scientific Medical Center" (for the period from 2019 to 2022).

Mesenchymal stem cells obtained from 19 patients with NOA, previously cultured at the Center for Cell Technology, Transplantation and Management, were injected into the same patients 2 weeks later during micro-TESE.

Research objectives:

1. Isolate mesenchymal stem cells from patients with non-obstructive azoospermia and study their phenotypic properties using flow cytometry.
2. To assess the safety of the route of introduction of mesenchymal stem cells into testicular tissue in patients with non-obstructive azoospermia.
3. To evaluate the regenerative effect of mesenchymal stem cells on the process of spermatogenesis in patients with non-obstructive azoospermia.
4. To study the therapeutic effects of mesenchymal stem cells on hormonal levels in patients with non-obstructive azoospermia.

Research methods:

1. Study of laboratory parameters and ultrasound of the scrotum (control).
2. Coagulogram (prothrombin index, prothrombin time, fibrinogen, APTT).
3. Hormonal profile (testosterone, FSH, LH, prolactin, inhibin B).
4. Analysis for karyotyping, microdeletion of the Y chromosome.
5. Study for tumor markers: CA 19-9, CYFRA, PSA (total and free), AFP, S-100, CEA, SCCA, CA 72-4.
6. ELISA: HBsAg, HBc.
7. Chest x-ray.
8. Ultrasound of the scrotum (localization of the testicles, the presence of inflammatory processes).
9. Ultrasound of the bladder, prostate, volume of residual urine (according to indications)

10. Testicular biopsy (number of spermatogonia, number of spermatocytes).
11. Histological examination of the convoluted tubules of the testis.
12. Evaluation of spermogram in dynamics after 6 months.
13. Statistical data processing.

Scientific novelty:

1. A new method of using autologous bone marrow mesenchymal stem cells in the treatment of secondary non-obstructive azoospermia has been developed.
2. The stimulating effect of autologous bone marrow mesenchymal stem cells on the appearance of spermatogenesis in patients with secondary non-obstructive azoospermia has been proven.
3. Autotransplantation of bone marrow mesenchymal stem cells revealed a decrease in FSH, an increase in testosterone and inhibin B levels.

Practical significance:

The use of autologous bone marrow mesenchymal stem cells is safe and regenerative effects on spermatogenic epithelium have been noted in patients with secondary non-obstructive azoospermia.

The main provisions for defense:

1. Autotransplantation of mesenchymal stem cells is a safe and effective procedure in the treatment of men with secondary non-obstructive azoospermia.
2. Autotransplantation of mesenchymal stem cells of the bone marrow causes an increase in testosterone, inhibin B; decrease in FSH levels.
3. After autotransplantation of bone marrow mesenchymal stem cells, activation of spermatogenesis was noted in patients with secondary non-obstructive azoospermia, which was confirmed by the detection of spermatozoa.

Conclusions:

1. In the resulting culture of bone marrow cells isolated from patients with secondary NOA, $1.5 \pm 0.3 \times 10^6$ MSC cells were obtained, of which 95% were viable with the phenotype CD73, CD90, CD105.
2. Transplantation of autologous bone marrow MSCs in patients with secondary NOA is a safe and well-tolerated procedure, as evidenced by the absence of significant complications during the follow-up period.
3. After the introduction of autologous bone marrow MSCs, the appearance of spermatozoa in patients with secondary NOA was noted.
4. Autotransplantation of mesenchymal stem cells reduced FSH levels from 28.62/21.72 (18.86; 29.8) to 14.56/13.08 (12.04; 16.2) $p < 0.001$; increase in testosterone levels from 6.82/4.88 (2.78; 9.2) to 10.04/7.87 (6.88; 11.26) $p = 0.002$; inhibin B from 7.22/5.4 (4.05; 9.31) to 17.52/16.7 (12.05; 23.65) $p < 0.001$.
5. The positive results of this work serve as the basis for continuing clinical research in the field of using cell technologies in the treatment of secondary non-obstructive azoospermia.

Practical recommendations:

A comprehensive examination with a mandatory assessment of the endocrine status is necessary.

More research is needed to replicate the experimental treatment in other centers that treat male infertility.

When preparing men for MSC autotransplantation with micro-TESE, it is necessary to use spermatogenesis-stimulating therapy with gonadotropins for 6 months.

Approbation of work:

The dissertation work was tested at an extended meeting of the Department of Urology and Andrology and the Department of Surgical Diseases with courses in angiosurgery and plastic surgery (Minutes No. 10/1 dated June 24, 2022).

The main results of the research and the provisions of the dissertation were reported in the speeches:

– at the XLIII International Scientific and Practical Conference at “EurasiaScience” February 15, 2022.

– at XII Global Science and Innovations 2021: Central Asia. International Scientific Practical Journal, Nur-Sultan, Kazakhstan, February.

– at the international conference “VII International Scientific and Practical Conference” Munich, Germany February 22 – 25, 2022

– at the XVII International Multidisciplinary Conference March (Madrid, 2022).

Publications:

Based on the materials of the study, 6 articles were published: one article was published in the journal Stem Cell Research and Therapy, which has the 90th percentile (Q1) and an article in the journal Urology, which has the 25th percentile (Q4) according to CiteScore in the Scopus database, 3 review articles in peer-reviewed domestic publications recommended by the Committee for Quality Assurance in Science and Education of the Ministry of Education and Science of the Republic of Kazakhstan and 1 article in a Russian journal. Also published 7 publications in the materials of international scientific and practical conferences (Kazakhstan, Russia, Canada, Madrid, Germany). There is 1 copyright certificate dated 05/22/2020 No. 10124.

The results of the work have been tested and implemented in uroandrological departments, in VFC in Astana.

Personal contribution of the dissertation student:

During the study, the author took part in determining the topics of the dissertation work, the formation of its methodological structure, the formulation of goals and objectives, the collection of research materials, independently conducted a statistical analysis and generalization of the results obtained, carried out clinical and laboratory interpretation of patient data, analysis of literary data on the topic of the dissertation work.

The author acted as an assistant in bone marrow sampling, culturing, changing nutrient media. Independently and as an assistant, she performed a testicular biopsy with the introduction of mesenchymal stem cells intratesticularly.

The author prepared and published the results of research in journals recommended by the Committee for Quality Assurance in Science and Education of the Ministry of Education and Science of the Republic of Kazakhstan, at international scientific and practical conferences and foreign publications.

Scope of structure and dissertation:

The dissertation materials are presented on 93 pages of typewritten text and include an introduction, literature review, description of materials and research methods, 5 sections, conclusions, practical recommendations, a list of references, including domestic and foreign sources, applications. The work is illustrated with 16 tables and 31 figures.