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Role of Stem Cells in Sertoli Cell Only Syndrome

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ABSTRACT

Background: The lack of germ cells in the seminiferous tubules is a hallmark of Sertoli cell-only syndrome (SCOS), a leading cause of male infertility. Recently, stem cell therapy has gained attention as a possible method for treating this illness.

Objectives: The aim of this study was to determine if stem cell treatment is effective for SCOS patients.

Subjects and Methods: From 2020 to 2022, 87 males had been diagnosed with primary infertility owing to SCOS. The patients were evaluated in a thorough clinical manner, which included testing of their genes, hormone profiles, and testicular biopsies, among other things. The testes were injected with pure stem cells that had been taken from bone marrow. Semen analysis, hormone levels, and testicular volume were periodically evaluated over the 12-month follow-up period.

Results: Following treatment, 69 patients (79.3% of the total) did not have sperm in their semen, whereas 18 patients (20.7% of the total) did. When compared to non-responders, respondents showed a statistically significant drop in FSH and LH levels and a rise in testosterone levels ($P < 0.001$). Hormonal changes were significantly different in the groups who responded and those that did not, and all responders had normal karyotyping and no AZF microdeletions.

Conclusion: Hormonal improvements were seen in responding individuals, suggesting that stem cell therapy may be a viable option for treating SCOS. Nevertheless, a significant number of patients chose not to participate, underscoring the need for more studies to improve patient selection and treatment procedures.

Keywords: Sertoli cell-only syndrome, hormonal profile, karyotyping, infertility, stem cell treatment.

INTRODUCTION

About 15% of couples attempting to conceive experience infertility, making it a common public health problem [1]. Nearly half of these instances are caused by male-related variables [2]. These factors mainly include problems with spermatogenesis, sperm quality (including motility and morphology), and sperm count. A very severe kind of male infertility, azoospermia occurs in 10-15% of instances and is defined by the lack of sperm in the ejaculate. Obstructive azoospermia (OA) occurs when the male reproductive system is obstructed, whereas nonobstructive azoospermia (NOA) is the outcome of substantial abnormalities in spermatogenesis induced by a variety of underlying causes [3].

Based on histological findings, NOA may be further classified as hypo spermatogenesis (HS), maturation arrest (MA), or Sertoli cell-only syndrome (SCOS) [4]. Between 26.3% and 57.8% of azoospermic males are affected with SCOS, which is characterized by seminiferous tubules that contain solely Sertoli cells [5,6]. While the precise origin of SCOS is still unknown, it has been linked to Y chromosome microdeletions, chromosomal abnormalities, undescended testis, radiation, cytotoxic medicines, and viral infections [7]. **Del Castillo *et al.*** initially recognized SCOS in 1947; other names for it include germ cell aplasia and Del Castillo syndrome. In this condition, normal Sertoli cells line the seminiferous tubules, but there are no germ cells present. Other symptoms include reduced testicular volume but otherwise normal secondary sexual traits. Complete SCOS, in which no germ cells are produced at all as a result of developmental problems, or localized SCOS, in which certain regions of spermatogenesis are normal, are the two main manifestations of this condition [8]. One possible method of conception for patients with focal SCOS is intracytoplasmic sperm injection (ICSI), which involves surgically removing spermatozoa [9].

The use of stem cell transplantation to treat male infertility caused by spermatogenic failures is showing great promise. In situations when the original spermatogonia are damaged or destroyed, stem cells have the ability to regenerate and differentiate into specialized cells, such as spermatogonial stem cells (SSCs). This means that stem cells may be able to repair spermatogenesis. Cancer patients and those with other spermatogenesis-affecting illnesses may benefit greatly from this approach [8]. The principal source of stem cells is bone marrow extraction, which can be painful. However, stem cells from this source are preferred for cell-based treatments due to their versatility, moderate immunogenicity, and ability to repair and regenerate tissues [9,10]. Inducing stem cells to develop into male or female germ cells may be achieved by techniques using growth factors, pharmacological agents, and genetic alterations [11]. Minor adverse effects, such as fever, sleeplessness, nausea, vomiting, or injection-site

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reactions, may occur with stem cell treatment and need close medical monitoring [12].

Studies have shown that stem cell transplantation into NOA-affected testes may start spermatogenesis, decrease apoptotic-related sterility, improve testosterone production, and may prevent antisperm antibody development [13]. Furthermore, transforming growth factor beta (TGF-) and bone morphogenetic proteins (BMPs) are secreted by these transplanted cells, which help patients regain biological functioning [14]. Local intratesticular stem cell injections as a means of stimulating spermatogenesis remain an area of ongoing investigation, despite the fact that many questions remain about their efficacy [15]. Stem cell treatment for SCOS patients is the focus of this investigation.

2. SUBJECTS AND METHODS

Study Design

The study ran from 2020 to 2022 and was conducted at two different locations: Benha University's Urology Department and Al-Azhar University's International Islamic Institute for Population Studies and Research's Andrology outpatient clinic. A total of eighty-seven male patients with a history of primary infertility attributed to Sertoli cell-only syndrome were included in the research.

Medical Evaluations

A comprehensive clinical examination was conducted on all participants, starting with a thorough history-taking session that focused on marital status, including the participants' ages, the length of their marriage, and any prior marriages. The purpose of the physical tests was to detect relevant illnesses such as dwarfism, gigantism, myxedema, and Klinefelter syndrome. In order to rule out blockages or congenital defects, a local examination of the testes was carried out to evaluate the testicular volume, consistency, existence of varicocele, and any abnormalities in the spermatic cord or vas deferens.

Analysis of Sperm

After a 2–7 day period of abstinence, the specimens were tested for sperm according to the protocols established by the World Health Organization (WHO). In order to confirm azoospermia in every patient, this study was vital.

Evaluations of Hormones and Histology

Researchers tested the subjects' testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels to assess their hormonal profile. To further assess the extent of spermatogenic arrest and look for histological signs of Sertoli cell-only syndrome (SCOS), testicular samples were also taken.

Genomic Evaluations

To detect chromosomal abnormalities like XX male syndrome or Klinefelter syndrome (47XXY), karyotyping was a part of the genetic examinations. Additionally, any deletions in the AZF area that may affect spermatogenesis were sought for using Y-chromosome microdeletion analysis, with an emphasis on loci A, B, and C.

Technology and Communication

Various specialized tools and media were used in the research to process and analyze samples. A laminar air flow cabinet was used to maintain a sterile environment. The OctoMACS system, developed by Miltenyi Biotec of Germany, was utilized for magnetic cell separation. Various microscopes were utilized for cell observation and counting. A centrifuge, a CO_2 incubator, and a water bath were also necessary equipment for the stem cell cultivation and blood component separation processes. Specific media, including MACS Buffer, Dulbecco's Phosphate Buffered Saline, and HiSep LSM 1077, were used in conjunction with sterile pipettes, culture tubes, and plates.

Methods for Collecting and Injecting Stem Cells

About 60 cc of iliac crest bone marrow aspirate was obtained under rigorous aseptic conditions. Before density gradient centrifugation, the aspirate was thinned with PBS and meticulously deposited onto Ficoll hypaque. Magnetic labeling with CD105 microbeads and FCR blocking antibodies was applied to the buffy coat, which contained mononuclear cells. After the stem cells were purified using the OctoMACS device, they were suspended in 1 ml of PBS. The cell concentration ranged from 3.7 to 5.2 million cells per cm^3, and the viability rate was 98.4-99.2%. Trypan blue was used to evaluate cell viability, and a hemocytometer was used for cell counting. Under local anesthetic, the stem cells that had been isolated were injected into the testicular cortex of both testes.

Follow-Up and Outcome Assessments

After receiving stem cell injections, patients were closely watched for a minimum of one year. Assessments of testicular volume, hormone profile (including FSH, LH, and testosterone), and semen analysis were performed every two months to track the efficacy of the stem cell treatment.

Statistical analysis

Version 20 of SPSS was used to conduct the statistical analysis. While number and percentage were employed to show nominal data, mean and standard deviation were utilized to express numerical data. Chi^2 test was used to compare nominal data. For normally distributed numerical data, independent t-test was used to compare between the 2 studied groups and paired t-test was used to compare before and after therapy in the same group. For abnormally distributed numerical data, Mann-Whitney test was used to compare between the 2 groups and Wilcoxon signed ranks test was used to compare

before and after therapy in the same group. P ≤0.05 was set as the significance level.

Ethical Approval:

This study was ethically approved by the Institutional Review Board of the Faculty of Medicine, Benha University and Al-Azhar University. All patients gave their written consent after receiving detailed information about the treatment and any potential risks or benefits. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

RESULTS

Table 1 examines the outcomes of stem cell treatment in patients who responded (n=18, 20.7%) and those who did not (n=69, 79.3%). Age, testicular size, and testicular testing results did not vary significantly across the categories. To highlight, although all successful patients had normal karyotypes, 15.9% of non-responders had Klinefelter syndrome (P = 0.070).

The successful group saw a noticeable drop in FSH of 45.81% compared to the non-responders of 20.32%, a decrease in LH of 26.84% compared to 18.76% (P = 0.01), and a rise in testosterone of 34.97% compared to 32.76% **(Table 1)**.

Table 1: Comparison of cases that responded well to stem cell treatment and those that did not regarding clinical, pre- and post-testicular biopsy, genetic background, and hormonal level.

Parameter	Failed (n=69, 79.3%)	Success (n=18, 20.7%)	P value
Age (Mean ± SD)	36.9 ± 7.35	34.83 ± 5.85	0.269
Testicular size before stem cells therapy			0.227
Normal (n, %)	35	12	
Small (n, %)	34	6	
Testicular examination before stem cells therapy			0.231
Normal (n, %)	61	17	
Varicocele (n, %)	7	0	
Cryptorchidism (n, %)	1	1	
Testicular biopsy before stem cells therapy			0.084
Primary spermatocyte (n, %)	9	2	
Secondary spermatocyte (n, %)	3	4	
Spermatid (n, %)	2	1	
SCOS (n, %)	55	11	
Karyotyping before stem cells therapy			0.070
Normal	58	18	
Klinefelter	11	0	
Microdeletion before stem cells therapy			0.779
Normal	65	18	
AZF a	1	0	
AZF b	2	0	
AZF c	1	0	
FSH (Mean ± SD)			
Before stem cells therapy	24.70 ± 5.2	22.07 ± 5.5	0.301
After stem cells therapy	19.68 ± 4.617	11.96 ± 2.97	< 0.001*
P value	0.016	< 0.001*	
% of changes	20.32	45.81	<`0.001*
LH (Mean ± SD) Before stem cells therapy	10.18 ± 2.75	7.03 ± 1.07	0.008*
After stem cells therapy	8.27 ± 1.679	5.143 ± 1.014	< 0.001*
P value	0.023*	< 0.001*	
% of changes	18.76	26.84	0.01*
Testosterone (Mean ± SD)			
Before stem cells therapy	2.367 ± 0.041	3.44 ± 0.66	< 0.001*
After stem cells therapy	3.52 ± 0.30	5.29 ± 0.77	< 0.001*
P value	0.041	< 0.001*	
% of changes	32.76	34.97	0.01*

FSH: follicle-stimulating hormone, LH: luteinizing hormone, *: significant p-value, SD: standard deviation, SCOS: Sertoli Cell-Only Syndrome, AZF: Azoospermia Factor

Table 2 offers a contrast between instances with Sertoli cell-only syndrome (SCOS) that responded well (n=11) and those that did not (n=55) after stem cell treatment. There was no statistically significant difference in patient age across the groups. Results from the two groups were statistically indistinguishable with regard to testicular size and pre-treatment assessment. Remarkably, there was no difference in the karyotypes of all successful patients compared to 20% of non-responders, who had Klinefelter syndrome.

There were noticeable shifts in hormone levels: FSH levels fell by 45.81% in successful cases compared to 20.32% in non-responders, LH levels fell by 26.84% compared to 18.76%, and testosterone levels rose by 34.97% compared to 32.76% **(Table 2)**.

Table 2: Comparison of hormonal levels and clinical and genetic contexts comparing SCOS patients who responded and those that did not.

Parameter	**Failed (n=55)**	**Success (n=11)**	**P value**
Age (Mean ± SD)	35.93 ± 6.45	35.09 ± 7.012	0.752
Testicular size before stem cells therapy			0.579
Normal (n, %)	30	7	
Small (n, %)	25	4	
Testicular examination before stem cells therapy			0.245
Normal (n, %)	48	10	
Varicocele (n, %)	6	0	
Cryptorchidism (n, %)	1	1	
Karyotyping before stem cells therapy			0.104
Normal	44	11	
Klinefelter	11	0	
FSH (Mean ± SD)			
Before stem cells therapy	23.2 ± 5.82	21.4 ± 5.2	0.13
After stem cells therapy	18.8 ± 4.9	11.2 ± 2.1	< 0.001*
P value	0.17	< 0.001*	
LH (Mean ± SD)			
Before stem cells therapy	10.4 ± 2.6	6.96± 1.98	0.009*
After stem cells therapy	8.4 ± 2.2	5.32 ± 1.2	< 0.001*
P value	0.017*	< 0.001*	
Testosterone (Mean ± SD) Before stem cells therapy	2.67 ± 0.1	3.54 ± 0.7	< 0.001*
After stem cells therapy	3.59 ± 0.2	5.4± 0.7	< 0.001*
P value	0.03*	< 0.001*	

*: significant p-value, FSH: follicle-stimulating hormone, LH: luteinizing hormone, SD: standard deviation, SCOS: Sertoli cell-only syndrome.

DISCUSSION

Caroppo and Colpi found that, with the exception of very unusual instances of hypogonadotropic hypogonadism, the spermatogenic failure-related Sertoli cell-only syndrome (SCOS) is incurable [15].

One kind of bone marrow cell is the fibroblast progenitor cell, which is also called SCs. These cells do not produce blood cells. The International Society for Cellular Therapy (ISCT) has established parameters that SCs must meet, including adhering to plastic surfaces and the capacity to differentiate into osteoblasts, adipocytes, and chondroblasts [16]. SCs primarily exert their therapeutic effects by modulating the immune system and communicating with neighboring cells via chemical signals; they typically have a modest capacity to elicit an immunological response [17,18]. There was no definitive evidence that SC injections might trigger tumor development, according to a meta-analysis by **Wang *et al.*** [12]. Also, we didn't find any major safety concerns. Several investigations have shown that SCs may undergo transdifferentiation and become spermatogenic cells under certain environmental circumstances. Mice who had SC transplants were able to make their own germ cells, according to a number of studies [19-21].

Depending on the situation, SCs may either integrate with neighboring cells at the site of injury, undergo cell type transformation, or secrete paracrine chemicals that entice local stem cells, all of which promote tissue regeneration [22,23]. Of the 87 individuals included in the trial, 20.7% produced sperm in their semen at various time intervals after receiving a single intratesticular injection of pure SCs. In contrast to the three sessions of intravenous subcutaneous infusions and local injections described in the case report by **Cassim and Mohamed** [24], our research only included a single injection into the testicles.

Hormone levels were positively affected by SC injection, leading to decreased FSH and LH levels and increased blood testosterone levels. According to the patient's medical history, the treated people showed increased testosterone levels, better sexual function and libido. On the other hand, when medicine was administered, there were significant differences in hormone levels between those who reacted well to therapy and those who did not. After looking at cases where the FSH level was twice the norm, it was shown that the initial FSH level was not a good predictor of success. All of the successful cases had a normal karyotype and no chromosomal abnormalities in the AZF region. This is a solid sign that things will turn out well. Although testicular biopsy findings might vary, all SCOS patients that have been successfully treated had normal karyotyping and no AZF microdeletions.

Another piece of evidence supporting SCs' excellent migration and transformation abilities into multiple cell types is the same success rate seen in SCOS cases compared to other non-obstructive azoospermia instances. So, it's not necessary to rule out stem cell treatment only because spermatogonia aren't present at all. Classic Klinefelter syndrome patients' hormonal profiles improved significantly, with lower FSH and LH levels and increased testosterone levels, but the men's sperm count was negative. The findings produced here are consistent with those of **Baghae *et al.*** [25]. In addition, neither the bone marrow aspiration nor the intratesticular injection processes were associated with any complications. After the subcutaneous injection, the patients were closely monitored.

The accumulation of genetic and epigenetic alterations poses hazards to the cells' safety, efficacy, and biological and therapeutic properties; so, this preventive step was taken to reduce such risks[26]. The pathogenic testicular alterations, including as scar tissue and microcalcifications, were seen in rams independent of the sperm retrieval method, according to research by **Fedder *et al.*** [27]. There was a correlation between these alterations and decreased blood flow as well. In addition, **Eliveld *et al.*** [28]. found that low testosterone levels might be transient (or "transient hypogonadism") for up to 26 months. Some individuals had smaller testicles, however only a tiny fraction of those people had erectile dysfunction, according to the research. Rather than hypogonadism, psychological factors including depression and worry were shown to be the main causes of erectile dysfunction in men who had unsuccessful sperm retrieval attempts.

There may not be enough people to draw any firm conclusions from this research, as the sample size is so tiny. The absence of a control group and observational design further complicate efforts to draw conclusions about the relationship between stem cell treatment and the results. Clinical tests and measures of testicular size rely on subjective evaluations, which might create bias. Also, the research doesn't take into consideration any problems or long-term repercussions that may come with stem cell therapy; these might affect how safe and effective the treatment is overall. Last but not least, therapy results may vary due to the lack of a consistent procedure for stem cell preparation and injection.

CONCLUSION

Hormonal improvements were seen in individuals who responded to stem cell therapy for SCOS, suggesting its potential as a treatment. Nevertheless, a significant number of patients chose not to participate, underscoring the need for more studies to improve patient selection and treatment procedures.

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