Original Paper

Mesenchymal stem cells restore ovarian function and follicular morphology changes in cyclophosphamide-induced infertility in female rats

Heba E. Sherif [1*], Yakout A.El- Senosi[1], Samy A. Hussein[1], Ehab M. ElZoghby[2], Mohamed A. Sobh[3], Heba Sheta[4], Basma H. Othman[5]

[1]*Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Benha University, Egypt.*
[2]*Department of Histology, Faculty of Veterinary Medicine, Benha University, Egypt.*
[3]*Urology and Nephrology Center, Faculty of Medicine, Mansoura University, Egypt.*
[4]*Department of Pathology, Faculty of Medicine, Mansoura University, Egypt.*
[5]*Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University, Egypt.*

ARTICLE INFO

ABSTRACT

Premature ovarian failure (POF) contributes significantly to female infertility in young women. It mainly developed after chemotherapy medication, particularly with alkylating cytotoxic such as cyclophosphamide (CYP). The present study was designed to determine the advantages of mesenchymal stem cells (MSCs) as therapy for POF conditions in female rat models. For POF induction, CYP (200mg/Kg b.wt) was injected intraperitoneally (i.p) followed by another activation dose of CYP (10mg/ b.wt, i.p) after one week. MSCs treatment was enhanced by Bone Marrow-derived stem cells (BM-MSCs) or Umbilical Cord-derived stem cells (UC-MSCs) after two weeks of POF induction. The normal control (NC) group rats were injected with saline. The effects of POF modeling and MSCs (1×10^6 cells) transplantation through tail-vein were examined by sex hormonal analysis and morphological examination of ovarian follicles. CYP-induced rats POF showed a significant reduction in AMH and E2 concentrations in addition to total, primordial, primary, secondary, and mature graffian follicles in contrast to the NC group (P<0.0001). The FSH and LH concentrations were increased in the CYP group and degenerated follicles than in the NC group (P<0.0001). Transplanting MSCs improved the histopathological and hormonal analysis of the ovarian microenvironment induced by CYP (P<0.0001) with no significant difference in response to BM-MSCs or UC-MSCs. It was concluded that MSCs injection was a possible strategy for improving CYP-induced morphological changes, and sex-hormonal alterations, potentially promoting the restoration of CYP-induced infertility via POF.

1. INTRODUCTION

Premature ovarian failure (POF) is one of the many reproductive system illnesses that can lead to female infertility, a condition that affects people worldwide (Gupta et al., 2022). Ovarian deterioration in young females is the major characteristic of POF, in addition to fluctuations in ovarian function before total cessation. Usually, amenorrhea, hypergonadotropism, and hypoestrogenism are present. Many risk factors, such as genetic defects, iatrogenic factors, chemotherapy injuries, autoimmune, and environmental and infectious causes, can result in POF (Li et al., 2021). Hormone replacement treatment (HRT) is effective in improving hot flashes, and vaginal and urinary symptoms in women with POF (Fu et al., 2021). To increase the effectiveness of POF treatment, innovative approaches such as ovarian transplantation, gene therapy, and stem cell therapy are desperately needed in addition to HRT (Wang et al., 2021; Guo et al., 2023). Mesenchymal stem cells (MSCs) have received increasing attention as a potential cell-based therapy, with several advantages over other cell sources, including greater abundance, fewer ethical considerations, and high capacity for self-renewal and differentiation (Guo et al., 2023). Clinical researchers have examined the therapeutic use of MSCs in female infertility. The clinical application of MSCs holds great promise for infertility treatment or ovarian insufficiency, and to improve reproductive health for a significant number of women worldwide (Esfandyari et al., 2020).

One of the most common causes of POF is chemotherapy, which is a major cancer treatment. After chemotherapy, POF affects about one-third of women under 40 years of age with cancer diagnoses (Chen et al., 2021). According to studies, cyclophosphamide can harm ovaries to varying degrees, which can lead to infertility (Ai et al., 2023). Chemotherapy in the ovaries can cause granulosa cells (GCs) and oocytes to undergo apoptosis. This can awaken dormant primordial follicles, which will cause the dormant follicle pool to fail. Follicle-stimulating hormone (FSH) increased, E2 decreased, and follicular abnormalities were observed by CYP injection in female rats. As a result, CYP is frequently employed to create experimental models of POF caused by chemotherapy (Abogresha et al., 2021; Li et al., 2021; İlgen et al., 2023; Wang et al., 2023). There is still no particular treatment for POF because of its complexity and specificity. Although there have been more restricted clinical trials, several stem cell sources have recently been employed and have shown encouraging outcomes in chemotherapy-induced POF models (Chen et al., 2021; Wang et al., 2021; Gupta et al., 2022).

---

* Correspondence to: Heba.sherif2705@gmail.com

Recently, there has been significant progress in stem cell technologies, providing hope for people with incurable diseases. Multipotent progenitor cells with the capacity for self-renewal and mesenchymal lineage development are known as mesenchymal stem cells or MSCs. Three major sources of MSCs—bone marrow (BM), adipose tissue (AT), or the umbilical cord (UC)—are used to treat human disorders. MSCs have been linked to treat a wide range of illnesses, such as skin burns, respiratory ailments, neurological disorders, metabolic/endocrine-related diseases, and cardiovascular conditions (Kim et al., 2021; Li and Wang, 2022; Hoang et al., 2022). MSCs treatment for POF may restore ovarian function, and strengthen the preclinical medical data supporting the use of MSCs in high-caliber clinical studies. Patients with POF now have hope thanks to the transplantation of MSCs, especially since the cells were obtained with less invasive isolation procedures and were presented ethically without controversy (Fu et al., 2021).

We created a rat POF *in vivo* model to assess the extent of CYP-induced damage to follicles and granulosa cells, involved in estrogen shortage and infertility. We also looked at how MSC transplantation helped to improve POF, which provided theoretical support for the clinical use of BM- and UC-MSCs.

2. MATERIAL AND METHODS

Approval Ethics

All experimental procedures were carried out following standards of national animal welfare. The Institutional Animal Care Committee recommendations and the Declaration of Helsinki's tenets were established (Approval No. BUFVTM- 11-10-2023) by the Benha University Institutional Animal Care Committee.

2.1. Experimental Animals.

The study used 200±20 g healthy, eight-week-old female Sprague-Dawley rats. The study was conducted at the Medical Experimental Research Center (MERC), Mansoura University, Egypt's Faculty of Medicine. The rats were inbred and kept in conventional laboratory circumstances with free access to food (regular pellet diet) and water, a temperature of $25 \pm 2°C$, a humidity of $60 \pm 5\%$, and a 12-hour light/dark cycle.

2.2. Chemicals, POF Model Establishment, and Experimental Design.

Cyclophosphamide (Cycram) was purchased from, KUP-EIMC pharmaceuticals company (Korea). ELISA kits determined all the hormones (SunLong Biotech Co., LTD, Zhejiang, China). MSCs identification monoclonal from (Biolegend, San Diego, California). Histopathological immune stains were purchased from (Abcam, Cambridge, Massachusetts) and (VECTOR Laboratories, CA, USA).
On the first day, intraperitoneal injection of cyclophosphamide (200 mg/kg) was administered, simulating acute exposure to a significant dose in cancer patients. An activation dose of 10 mg/kg was given one week later (Abogresha et al., 2021). To validate the establishment of the chemotherapy-induced POF rat model, a pilot group consisting of four rats was slaughtered before the commencement of the investigation. One week following induction, atrophic alterations were seen in the ovarian tissues.
Four groups of forty rats (10 rats each) were recruited: Saline was given to the rats in the Normal Control (NC) group

during the trial; the POF group: received an intraperitoneal injection of CYP to induce POF; Rats treated with MSCs from bone marrow (BM-MSCs, 1×10^6 cells) and Umbilical Cord treated rats (UC-MSCs, 1×10^6 cells) injected into the tail vein (Wang et al., 2020), both after concomitant induction of POF using CYP (CYP-BM group) and (CYP-UC group). At 7 and 14 days after MSCs treatment, rats were anesthetized and sacrificed; blood from direct cardiac puncture was used to obtain serum samples for hormone analysis. For immunohistochemical staining and histological analysis, ovarian tissue was obtained.

2.3. Female Rat MSCs (BM and UC) Isolation and Identification.

A 5-week-old healthy Sprague-Dawley rat was used to isolate BM-MSCs from its tibia and femur (Sobh, 2014). Pregnant rats were used to isolate the UC-MSCs (Zhang et al., 2018). The full-term umbilical cord (UC) was retrieved before rat delivery. After manually dividing the isolated UC into three sections measuring 1-2 mm, it was incubated for 30 minutes at 37°C with a gentle agitation with 0.075 % collagenase type II followed by 0.125 % trypsin.
Both MSCs (BM and UC) were cultured in low glucose Dulbecco's modified Eagle's medium (L-DMEM) (Capricorn-Scientific, Germany) supplemented with 10% BM and 5% UC fetal bovine serum (Thermo Fisher, Carlsbad, CA), 100 U/mL penicillin, and 100 μg/mL streptomycin. A 1×10^6 cells/mL density was seeded into a culture flask and cultured in a humidified incubator at 37 °C with 5% CO2. The adherent cells were recognized as MSCs after a medium replacement 48 h of culture. Passages three through seven of MSCs with a consistent morphological appearance as long, orderly spindles resembling fibroblasts were employed in all investigations.
Oil Red O staining confirmed adipogenic differentiation of cellular aggregates in the BM-MSCs and UC-MSCs cultures by cultivating them in the adipogenic induction medium (Biolegend, San Diego, California). MSCs surface marker expression was measured by a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA) using CD29-PCYNA, anti-Sca-PE as positive markers, CD34- PCYNA, and TER119-PE as negative markers (all from Biolegend, San Diego, California). To transplant BM-MSCs or UC-MSCs into POF rats, 1×10^6 cells third-passage in 100 μL of PBS was injected into the tail vein (Zhang et al., 2018). The control group of rats received a 100 μL injection of PBS.

2.4. Serum hormones analysis.

Serum hormone profiles included anti-Müllerian (AMH), estradiol (E2), follicular stimulating (FSH), and luteinizing hormone (LH) were examined to validate the diagnosis of CYP-induced infertility. For determination of their levels in serum, blood samples were collected from rats by heart puncture while anesthetized. The serum was separated by centrifugation at 4000 rpm for 10 minutes. The hormone concentrations were determined according to the manufacturer's instructions, ELISA kits (SunLong Biotech Co., LTD, Zhejiang, China).

2.5. Histopathological Examination and Immuno-histochemistry Staining.

The ovary specimens were treated in graded ethyl alcohol to paraffin blocks after they were preserved in 10% formaldehyde for 24 hours. The blocks were sectioned at 4-5 μm thick, to perform histological and immunohistochemical examination methods. Hematoxylin and eosin (H&E)-stained ovarian sections were analyzed quantitatively to determine normal developing and degenerated follicles in

three sections per ovary (in randomly selected areas) in addition to the overall architecture and histopathological alterations. The primordial follicles had small central oocytes surrounded by a single layer of flat granulosa cells (GCs); the primary follicles had a larger oocyte, surrounded by a single layer of GCs, and outer flat stromal cells. An oocyte with zona pellucida (ZP) covering, corona radiata (CR) cells, GCs, and one or more tiny fluid holes was seen in the secondary or antral follicles. The largest follicles were Graafian follicles (GF), located near the ovarian surface. They had a cumulus oophorus (CO), a sizable fluid chamber, and GCs lined their walls in several layers. With the CO tilted to one side, layers of GCs, ZP, and CR encased a sizable oocyte.

Positively charged slides were utilized in the manual immunohistochemical labeling of caspase-3, the crucial mediator of programmed cell death (apoptosis) is frequently activated death protease. Sections were treated with 3% H_2O_2 for 10 min and washed with PBS for 5 min. The primary caspase-3 antibodies (Anti-caspase-3 (ab238936), IHC-P, Abcam, Cambridge, Massachusetts) at a dilution of 1:200 were incubated overnight at 4 °C. Sections were again washed with PBS. Biotinylated secondary antibodies (PK-7800, VECTOR Laboratories, CA, USA) were applied to slides for 30 min at room temperature. Subsequently, hematoxylin was used to counterstain the sections after power-staining with TM 1.0 poly horseradish peroxidase DAB. Examining 3 random sections per ovary, the proportion of color area occupied by the brown-stained cytoplasm with anti-caspase 3 was computed.

2.6. Statistical Analysis.

Continuous variables were represented as the mean ± standard error mean (SEM). The data were examined using the T-test comparing groups and analysis of variance (ANOVA) followed by Bonferroni's post hoc analysis was used for comparisons between groups. The significant difference p value less than 0.05 ($p < 0.05$) was considered statistically significant. The statistical software package SPSS for Windows (Version 20; SPSS Inc., Chicago, IL, USA) was used.

3. RESULTS

3.1. Identification of MSCs.

The results of FCM indicated that the MSCs express positive mesenchymal progenitor markers with anti-Sca and CD29, and negative mesenchymal markers with CD34 and TRE119 population cells. The purity of the isolated cells reached over 98% (Fig 1). MSCs can differentiate into different mesoderm-type cells, and the adipocyte differentiation was documented with Oil Red O stains of cellular aggregates.

3.2. Ovarian function restoration after MSCs transplantation.

Statistically significant decreased concentrations in both AMH and E2 were observed in the CYP group compared to the NC group (p<0.0001, Table 1). Improved AMH and E2 concentrations were found in both MSCs groups CYP-UC and CYP-BM (p<0.0001), to a level comparable to the NC group. On the other hand, statistically significant increases in both FSH and LH concentrations in the CYP group more than in the NC (p<0.0001). This increase was a retreat with both MSCs groups CYP-UC and CYP-BM to the level resample to the NC group (p<0.0001, Table 1).
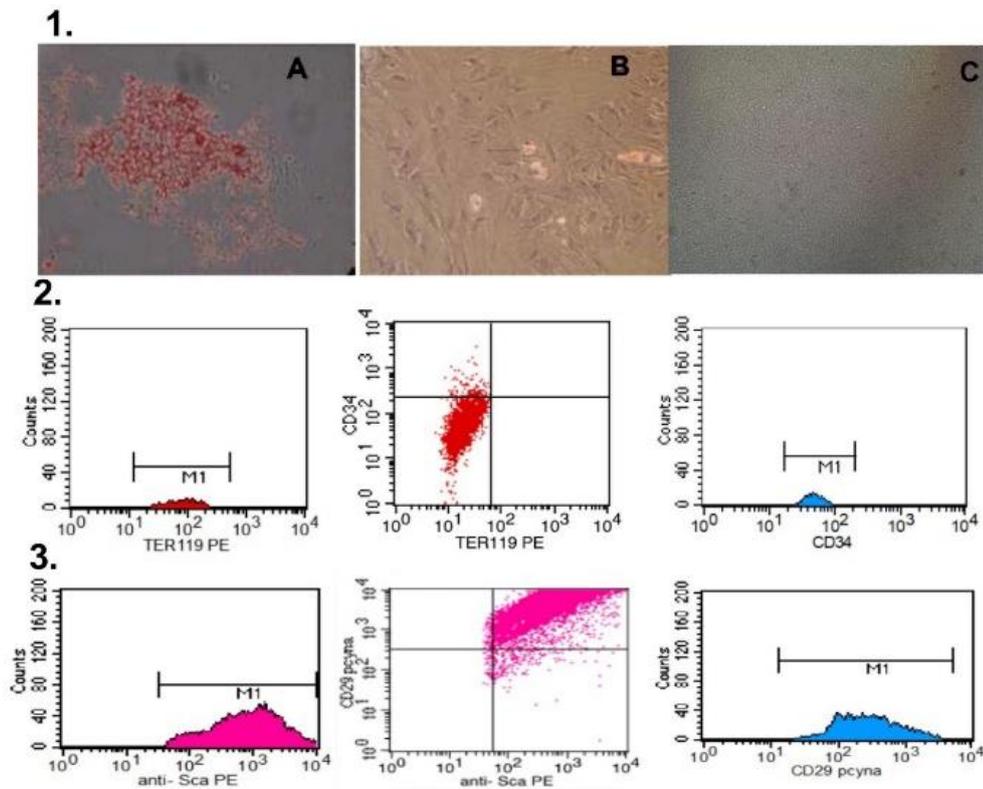

Figure (1): Isolation and identification of MSCs. 1. Oil Red O stain showed adipogenic differentiation of cellular aggregates (A) in the bone marrow BM-MSCs cultures and (B) lipid droplet stained with ORO in UC-MSCs cultures. (C) The isolated MSCs exhibited typical fibroblast-like morphology. Bar: 100 μm. **2.** FCM results of negative marker expression for MSCs with CD34 and TRE119 population cells. **3.** FCM results of positive marker expression for MSCs with anti-Sca and CD29 population cells.

3.3. MSCs transplantation improves female rats' ovarian architecture.

Fourteen days after modeling/injection, we examined the anatomy of the ovary using HE staining and counted all follicles in four groups of rats, accordingly (Fig 2). A notable reduction in the quantity of distinct follicular phases was seen in the CYP group compared to the NC group (p<0.001). The average number of follicles restored after MSCs transplantation in both CYP-UC and CYP-BM groups compared to the CYP group (p<0.0001), though it did not reach the level of the NC group (p<0.0001). These findings suggest that MSCs transplantation may partially restore ovarian structure and function (Fig 2). The number of degenerated follicles was greater in the CYP group than in the NC group (Fig 2). These follicles were restored to the level observed in the NC group following MSCs therapy (p<0.0001).

The normal follicular structure is shown in Fig (3). The primordial, primary, secondary, and mature Graafian follicles were observed in female rats in the NC group. Fig (4) shows ovarian damage with degenerated Graafian follicles, dilated congested blood vessels, thick-walled blood vessels, interstitial edema, and inflammatory infiltrate. With higher magnification, the photomicrograph shows ovarian damage with degenerated Graafian with degenerated lining and absent primary oocyte. Fig (5) shows partial restoration of ovarian architecture in the BM-MSCs group with a significant decrease in vascular congestion, edema, and inflammatory cellular infiltrate. There is also a detectable degenerated Graafian follicle. In the UC-MSCs group, a remarkable improvement of the ovarian tissue from that shown in the POF group with the restoration of ovarian follicular differentiation and detection of multiple mature Graafian follicles. With higher magnification, the photomicrograph shows mature Graafian and secondary ovarian follicles with primary oocytes inside.

Fig (6) depicts ovarian sections stained with caspase-3 from the four groups. The NC group exhibited a lack of immunoreactivity towards caspase-3 in the stroma, theca cells, the granulosa, and oocyte of Graafian follicles. The CYP group demonstrated Graafian follicles with strong positive immunological reactions in the granulosa and theca cells and an extensive positive stain with the oocyte and the stroma. The group that received MSCs exhibited Graafian follicles, characterized by a few positive immune stains on the granulosa and theca cells, while the oocyte and stroma displayed faint positive stains.
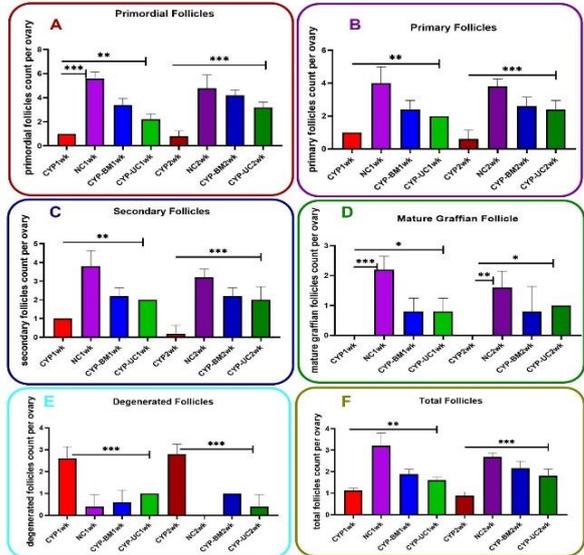

Figure (2): Effect of MSCs transplantation on follicular development. Ovarian H&E staining sections were from NC, CYP control groups, BM-MSCs, and UC-MSCs transplantation groups (by tail vein injection). Follicles were numbered and classified. Data were means ± SEM of counts for different stages of follicles in three experiments. *p<0.01; **p<0.001; ***p<0.0001. the comparison was done against the CYP group, after 1 and 2 weeks.
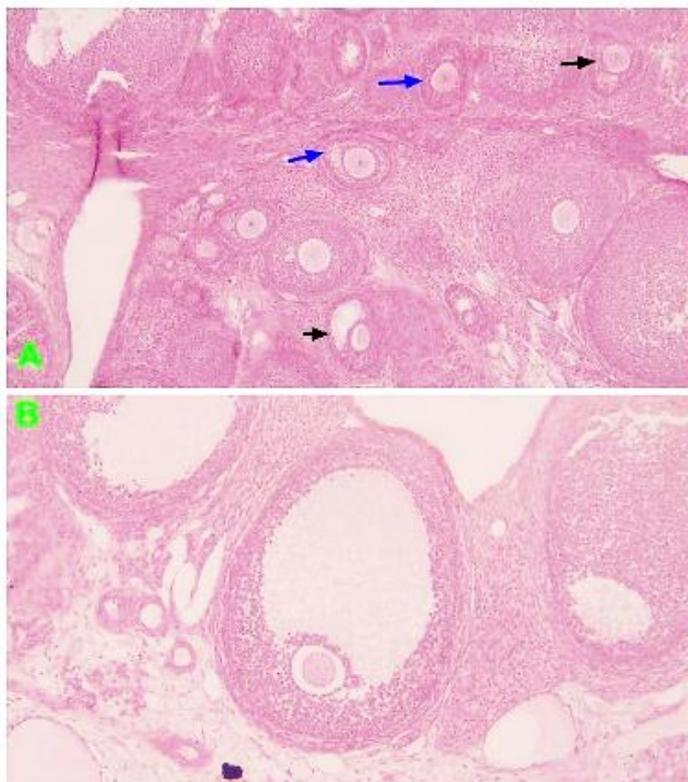

Figure 3: A- Photomicrograph from the control group showing different stages of normal follicular development with little stroma in between. Primary follicles (blue arrows) and secondary follicles (black arrows). (Hematoxylin and Eosin; 10x). B- Mature Graafian follicles (H & E; 40x).
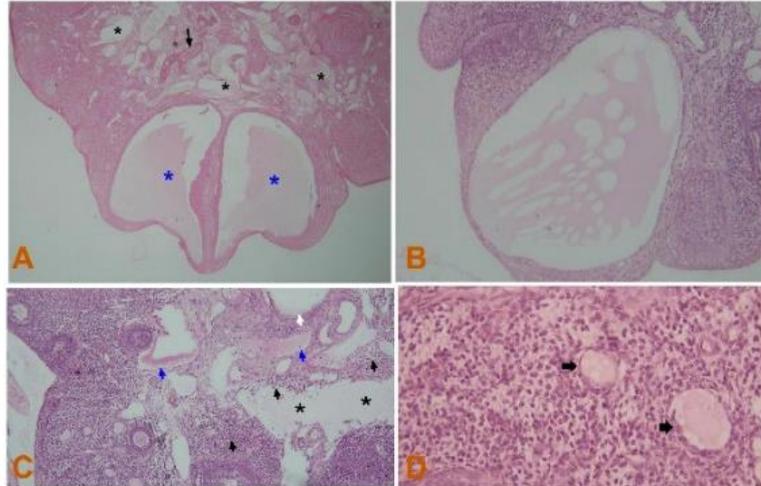
Figure (4): A- Photomicrograph from POF Group showing ovarian damage with degenerated Graafian follicles (blue Asterix), dilated congested blood vessels (black Asterix), thick-walled blood vessels (arrows), and interstitial edema (H & E; 10x). B- Ovarian damage with degenerated Graafian follicles (H & E; 20x). C- Partial ovarian damage with degenerated ovarian follicles (white arrows), dilated congested blood vessels (blue arrows), thick-walled blood vessels, interstitial edema (Asterix), and inflammatory infiltrate (black arrows), (H & E; 10x). D-Ovarian damage with degenerated ovarian follicles (arrows) with degenerated lining and absent primary oocyte (H & E; 40x).
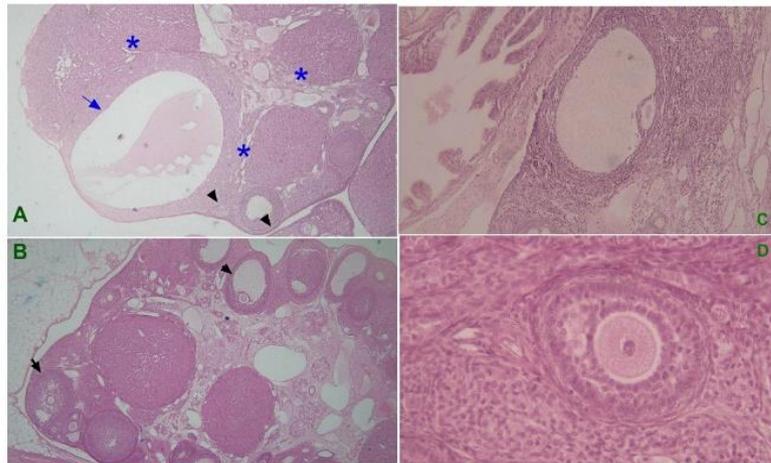

Figure (5): A- Photomicrograph from BM-MSCs group showing partial restoration of ovarian architecture with a significant decrease in vascular congestion, edema, and inflammatory cellular infiltrate. Multiple corpora lutea (Asterix), and primary and secondary ovarian follicles (black arrows) exist. There is also a degenerated Graafian follicle (blue arrow) (H & E; 10x). B- Photomicrograph from UC-MSCs group showing remarkable improvement of the ovarian tissue from that shown in the POF group with restorating ovarian follicular differentiation and detecting multiple mature Graafian follicles (black arrows) (H & E; 10x). C- mature Graafian follicles (H & E; 20x). D- mature secondary ovarian follicles with primary oocytes inside (H & E; 40x).

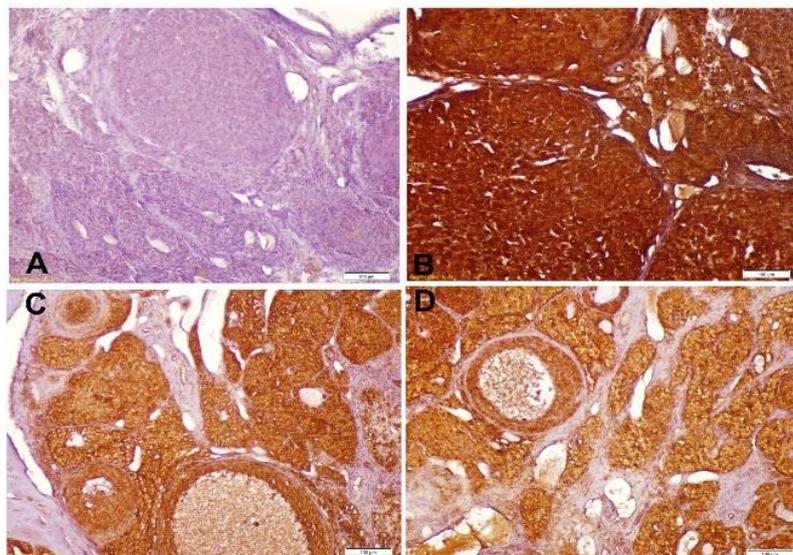Figure (6): Ovarian cell apoptosis was investigated by evaluating caspase 3 expression by immunohistochemistry analysis.Photomicrographs of anti-Caspase 3 immune reactions (IHC staining X 40 A, B; X 10 C, D) in ovarian sections (A–D). (A) The NC group with no immune stain (B) the CYP group showing dense immune reactions (C) the BM-MSCs group and (D) the UC-MSCs group, both showing moderate immune stains.

Table 1 Effect of MSCs treatment on serum AMH, E2, FSH, and LH concentrations in cyclophosphamide-induced premature ovarian failure in female rats.

Animal groups	AMH (ng/ml)		E2 (pg/ml)		FSH (ng/ml)		LH (ng/ml)	
	1st week	2nd week	1st week	2nd week	1st week	2nd week	1st week	2nd week
G1: CYP	2.72 ± 0.14	2.32 ± 0.15	34.90 ± 6.04	30.86 ± 1.69	9.08 ± 0.27	9.48 ± 0.49	5.58 ± 0.27	5.94 ± 0.28
G2: NC	7.66 ± 0.29***	7.94 ± 0.12***	60.88 ± 1.33*	64.74 ± 3.24***	5.06 ± 0.21***	5.04 ± 0.35***	2.91 ± 0.19***	2.80 ± 0.11***
G3: CYP+ BM-MSCs	4.80 ± 0.25**	5.16 ± 0.22***	45.99 ± 3.44	52.20 ± 3.01**	7.62 ± 0.26**	7.24 ± 0.24**	3.88 ± 0.22**	3.26 ± 0.12***
G4: CYP+ UC-MSCs	5.58 ± 0.42***	6.20 ± 0.19***	58.32 ± 6.05**	65.88 ± 6.06**	6.76 ± 0.33**	6.24 ± 0.28**	3.10 ± 0.24***	2.80 ± 0.11***
F	39.63	95.03	2.52	14.38	31.71	34.94	24.93	70.42

Data represented by the mean ± SEM. SEM: Standard error of the mean. *$p<0.05$; **$p<0.001$; ***$p<0.0001$. The comparison was made against the CYP group. CYP: cyclophosphamide; NC: Normal Control; BM-MSCs: Bone Marrow- mesenchymal stem cells treatment; UC-MSCs: Umbilical Cord- mesenchymal stem cells treatment (UC-MSCs), AMH: Anti-Müllerian Hormone; E2: Estradiol; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; F: ANOVA Factors between groups.

4. DISCUSSION

Presently, infertility stands as a fundamental determinant impacting the well-being of women. Ovarian dysfunction and early ovarian reserve depletion are symptoms of POF, a multifactorial disease with an unknown origin that is among the primary causes of infertility, especially among women under the age of 40 years (Chen et al., 2021). The resulting outcomes were obtained from our investigation in which BM-MSCs and UC-MSCs were implanted into the ovaries of POF rats via the tail vein revealed that MSCs transplantation ameliorates ovarian function, restores ovarian architecture, and conserves serum hormone concentrations. Despite the critical role that chemotherapeutic medicines play in the fight against cancer, the alarming number of adverse effects associated with these medications is cause for concern. As an alkylated agent, cyclophosphamide is a first-line treatment for numerous cancers and immunosuppressant medications (Voelcker, 2020) and carries the greatest POF risk. Due to the induction of CYP resistance and the activation of specific survival signaling pathways, the therapeutic efficacy is limited to 15–20 percent of patients. Therefore, to overcome this resistance, it is imperative to escalate the dosage, albeit with adverse effects on non-target tissues that are not intended, such as hepatotoxicity, nephrotoxicity, and reproductive toxicity (İlgen et al., 2023; Wang et al., 2023). Similarly, the current study involves the induction of serious damage to ovarian function and architecture by interpersonal injection of the CYP rats. The capacity of stem cells to restore damaged tissues is enormous. Pluripotent cells have emerged as a crucial therapeutic choice in regenerative medicine technology, owing to their utilization in tissue engineering-based approaches to human degenerative disorders. It has been demonstrated that numerous types of stem cells can promote recovery in the treatment of POF (Chen et al., 2021; Wang et al., 2021; Gupta et al., 2022). Similarly, bone marrow and umbilical cord mesenchymal stem cells were utilized in the current investigation. A single cell suspension was subsequently produced and administered via tail vein infusion into female rats injured with the CYP to induce POF. There are no documented cases of tumor development following MSC therapy and a documented ovarian function and morphology was achieved. For ovarian dysfunction, MSCs can directly and impulsively migrate to the injured ovary and survive there under the stimulation of multiple factors, which facilitates ovarian recovery (Fu et al., 2021). The number of differentiated and functionally integrated MSCs is too small to explain the observed improvements in ovarian function. Furthermore, whether MSCs differentiate into oocytes after migrating to injured tissue is still unknown. Improved ovarian function in these studies might be driven by paracrine mechanisms. These mechanisms involve the secretion of certain cytokines, including vascular endothelial growth factor, insulin-like growth factor, and hepatocyte growth factor, which are helpful for angiogenesis, anti-inflammation, immunoregulation, antiapoptosis, and antifibrosis to help ovarian restoration (Zhao et al., 2019). Intraperitoneal administering of CYP induces significant alterations in reproductive hormone concentration in the CYP group as compared to the NC group. The primary ovarian hormones that regulate folliculogenesis are FSH and LH. Furthermore, granulosa cells (GCs) secrete anti-Müllerian hormone (AMH) during the primordial follicle phase and initial phase of antral follicles. As a result, AMH plus estrogen has a negative effect on follicular growth and influences FSH levels (Fu et al., 2021). Once GCs are disrupted through chemotherapy or other treatments, estrogen and AMH levels decrease, causing FSH to rise and the follicular pool to be depleted (Williams and Erickson, 2015). POI is detected by a decrease in estrogen and AMH secretion and a rise in FSH secretion. Insights into the mechanisms underlying ovarian dysfunction and follicular pool depletion can thus aid in the identification and development of effective treatments for ovarian dysfunction (Domniz and Meirow, 2019). With MSCs transplanted via tail vein injection heals these conditions by improving hormonal disorders and restoring ovarian function. The findings of this study demonstrated that transplantation of MSCs into rats with POF can have preventative and therapeutic effects. BM-MSC and UC-MSC transplantation improved ovarian function, as reported by previous research (Wang et al., 2020; Yang et al., 2020; Zhang et al., 2021; Wang et al., 2023). Several studies have shown the beneficial effects of bone marrow stromal cell treatment in a chemotherapy-induced ovarian failure animal model. Specifically, the results showed that ovarian structure and functions could be restored by bone marrow stromal cells (Mohamed et al., 2018). Although chemotherapy drugs can inhibit the growth of tumor cells, they can also lead to granulosa cell apoptosis, follicular atresia, ovarian function decline, and other manifestations of premature ovarian failure. Granulosa cells, which are located on the lateral side of the oocyte zona pellucidum and secrete estrogen under the action of follicle-stimulating hormone and other paracrine factors, play a role in nutrition and support of oocytes. Granulosa cell apoptosis thus leads to a decrease in estrogen levels in the body, affecting the normal development of oocytes. Abd-Allah et al. (2013) used bone marrow stromal cells from male rabbits to treat cyclophosphamide-induced ovarian failure and discovered that the ovarian functional reserve and number of follicles were improved. In addition, there were increased estrogen and vascular endothelial growth factor levels, reduced follicle-stimulating hormone levels, and diminished caspase-3 expression. Badawy et al.

(2017) showed that bone marrow stromal cells were able to restore ovaries damaged by chemotherapy in mice. Subsequent examination by HE staining revealed that the loss of several ovarian follicle types in rats was substantially mitigated following MSC transplantation. While the ovarian structure and function nearly returned to normal levels, a notable deficiency was observed in reaching the healthy rats, suggesting that mesenchymal stem cells had a protective effect on ovarian tissue structure and function. It is suggested that once mesenchymal stem cells (MSCs) from either bone marrow (BM) or umbilical cord (UC) reach the injury site, they secrete extracellular vesicles or exosomes, which trigger neighboring MSCs to help repair damaged follicles. In light of the CYP-induced hyperactivation of primordial follicles in rats, we investigated the proliferation and apoptosis of ovarian tissue from CYP-induced rats. As reported in prior research, the outcomes demonstrated that both MSCs types significantly inhibited apoptosis in follicles, including primordial follicles, and impeded the proliferation of primordial follicles in rats. This allowed for the retention of a greater number of follicles in rat ovarian tissue, thereby mitigating the CYP-induced damage to rat oocyte tissue (Yang et al., 2020; Zhang et al., 2021; Ai et al., 2023). Both types of MSCs were found to prevent programmed cell death, or apoptosis, as evidenced by the expression of caspase-3 in the ovarian tissue. The CYP group showed an overexpression of caspase-3, which was subsequently reduced after treatment with MSCs derived from either bone marrow (BM) or umbilical cords (UC). These findings are consistent with other research studies that have also identified the role of caspase-3 in ovarian tissue damage following CYP treatment, albeit with different treatment protocols (Abdel-Aziz et al., 2020; Abogresha et al., 2021; Al-Shahat et al., 2022). Additionally, UC-MSCs have been used in several animal models to successfully treat POF by reducing apoptosis of granulosa cells, decreasing follicle-stimulating hormone serum levels, and increasing estrogen and anti-Mullerian hormone levels (Mohamed et al., 2019). Elfayomy et al., (2016) proposed that UC-MSCs could reverse paclitaxel-induced apoptosis of ovarian cells either by establishing a normal arrangement of the surface epithelium and tunica albuginea, or by upregulating cytokeratin 8/18, transforming growth factor-β, and proliferating cell nuclear antigen to suppress caspase-3 expression.

5. CONCLUSIONS

POF is the term used to refer to the condition where ovarian function declines to an abnormal level in women before the age of 40. This condition has the potential to result in issues such as infertility. In summary, our research provides the foundation that mesenchymal stem cell (MSCs) administration promotes hormone secretion and inhibits apoptosis and inflammation in granulosa cells, hence facilitating the restoration of the POF condition. According to our findings, individuals with acute POF triggered by CYP may use MSCs-based treatment as an alternative therapy which provides support for future clinical trials using MSC transplantation.

6. REFERENCES

1. Abd-Allah S.H., Shalaby S.M., Pasha H.F., El-Shal A.S., Raafat N., Shabrawy S.M., Awad H.A., Amer M.G., Gharib M.A., El Gendy E.A., et al. 2013. Mechanistic action of mesenchymal stem cell injection in the treatment of chemically induced ovarian failure in rabbits. Cytotherapy, 15,1 , 64–75.

2. Abdel-Aziz A.M., Mohamed A.S.M., Abdelazem O., Okasha A.M.M., Kamel M.Y. 2020. Cilostazol protects against cyclophosphamide-induced ovarian toxicity in female rats: role of cAMP and HO-1, Toxicology Mechanisms and Methods, 30,70 , 526-535.

3. Abogresha N.M., Mohammed S.S., Hosny M.M., Abdallah H.Y., Gadallah A.M. Greish, S.M. 2021. Diosmin Mitigates Cyclophosphamide Induced Premature Ovarian Insufficiency in Rat Model. Int. J. Mol. Sci., 22, 3044.

4. Ai G., Meng M., Guo J., Li C., Zhu J., Liu L., Liu B., Yang W., Shao X., Cheng Z., et al. 2023. Adipose-derived stem cells promote the repair of chemotherapy-induced premature ovarian failure by inhibiting granulosa cells apoptosis and senescence. Stem Cell Research & Therapy, 14, 75.

5. Al-Shahat A., Hulail M.A.E., Soliman N.M.M., Khamis T., Fericean L.M., Arisha A.H., Moawad R.S. 2022. Melatonin Mitigates Cisplatin-Induced Ovarian Dysfunction via Altering Steroidogenesis, Inflammation, Apoptosis, Oxidative Stress, and PTEN/PI3K/Akt/mTOR/AMPK Signaling Pathway in Female Rats. Pharmaceutics, 14, 2769.

6. Badawy A., Sobh M.A., Ahdy M., Abdelhafez M.S. 2017. Bone marrow mesenchymal stem cell repair of cyclophosphamide-induced ovarian insufficiency in a mouse model. International Journal of Women's Health., 9,441–447.

7. Chen H., Liu C., Zhu S., Li S., Zhang Q., Song L., Liang X. 2021. The Therapeutic Effect of Stem Cells on Chemotherapy-Induced Premature Ovarian Failure. Curr Mol Med., 21,5 , 376–384.

8. Domniz N. and Meirow D. 2019. Premature ovarian insufficiency and autoimmune diseases. *Best Pract. Res. Clin. Obstet. Gynaecol.,* 60,42–55.

9. Elfayomy A.K., Almasry S.M., El-Tarhouny S.A., Eldomiaty M.A. 2016. Human umbilical cord blood-mesenchymal stem cells transplantation renovates the ovarian surface epithelium in a rat model of premature ovarian failure: possible direct and indirect effects. Tissue & Cell*.,* 48,4 , 370–382.

10. Esfandyari S., Chugh R.M., Park H.S., Hobeika E., Ulin M., Al-Hendy A. 2020. Mesenchymal Stem Cells as a Bio Organ for Treatment of Female Infertility. *Cells.,* 9,10 ,2253.

11. Fu Z.J., Li X.D., Wei D., Ding X.L. 2021. Clinical Application Potential of Umbilical Cord Mesenchymal Stem Cells in Chemotherapeutic Ovarian Failure. Reproductive and Developmental Medicine, 5, 55- 62.

12. Guo C., Ma Y., Situ Y., Liu L., Luo G., Li H., Ma W., Sun L., Wang W., Weng Q., et al. 2023. Mesenchymal stem cells therapy improves ovarian function in premature ovarian failure: a systematic review and meta-analysis based on preclinical studies. Front. Endocrinol., 14,1165574.

13. Gupta S., Rajput M., Sultan T., Solanki B., Rasheed N., Vishnoi V., Thakur N., Jaineeta, John L., Khan S., et al. 2022. Using Mesenchymal Stem Cells to Treat Female Infertility: An Update on Female Reproductive Diseases A Review Article. Neuro Quantology, 20 ,7 , 2424- 2432.

14. Hoang D.M., Pham P.T., Bach T.Q., Ngo A.T.L., Nguyen Q.T., Le P.T.T., Hoang V.T., Forsyth N.R., Heke M., Nguyen L.T. 2022. Stem cell-based therapy for human diseases. Signal Transduction and Targeted Therapy,7, 272.

15. İlgen O., Yılmaz F., Kurt S. 2023. Protective and/or therapeutic effects of berberine in a model of premature ovarian failure induced by cyclophosphamide in rats. Turk J Obstet Gynecol., 20, 227-33.

16. Kim K.S., Bou-Ghannam S., Kameishi M., Oka D.W., Grainger T.O. 2021. Allogeneic mesenchymal stem cell sheet therapy: A new frontier in drug delivery systems. Journal of Controlled Release, 330, 696-704.

17. Li C. and Wang B. 2022. Mesenchymal Stem/Stromal Cells in Progressive Fibrogenic Involvement and Anti-Fibrosis Therapeutic Properties. Front. Cell Dev. Biol., 10, 902677.

18. Li S., Liu M., Ma H., Jin Q., Ma Y., Wang C., Ren J., Liu G., Dai Y.2021. Ameliorative effect of recombinant human lactoferrin on premature ovarian failure in rats after cyclophosphamide treatments. Journal of Ovarian Research, 14,17.

19. Mohamed S.A., Shalaby S.M., Abdelaziz M., Brakta S., Hill W.D., Ismail N., Al-Hendy A. 2018. Human mesenchymal stem cells partially reverse infertility in chemotherapy-

induced ovarian failure. Reproductive Sciences, 25,1 , 51–63.

20. Mohamed S., Shalaby S., Brakta S., Elam L., Elsharoud A., Al-Hendy A. 2019. Umbilical cord blood mesenchymal stem cells as an infertility treatment for chemotherapy-induced premature ovarian insufficiency. Biomedicines, 7,1 ,7.

21. Sobh M.A. 2014. Adipogenesis of Sprague Dawley rats mesenchymal stem cells: a morphological, immunophenotyping and gene expression follow-up study. Anat Cell Biol., 47,83-90.

22. Voelcker G. 2020. The Mechanism of Action of Cyclophosphamide and Its Consequences for the Development of a New Generation of Oxazaphosphorine Cytostatics. Sci. Pharm., 88, 42.

23. Wang J., Liu W., Yu D., Yang Z., Li S., Sun X. 2021. Research progress on the treatment of premature ovarian failure using mesenchymal stem cells: A literature review. Frontiers in Cell and Developmental Biology, 9, 749822.

24. Wang X., Yuan P., Zeng M., Sun M., Wang X., Zheng X., Feng W. 2023. Allantoin Derived From Dioscorea opposita Thunb Ameliorates Cyclophosphamide-Induced Premature Ovarian Failure in Female Rats by Attenuating Apoptosis, Autophagy, and Pyroptosis. Cureus, 15,12 , e50351.

25. Wang Z., Wei Q., Wang H., Han L., Dai H., Qian X., Yu H., Yin M., Shi F., Qi, N. 2020. Cell Therapy Cord in a Rat Model of Autoimmune-Induced Failure. Cells International, 2020, 3249495.

26. Williams C.J. and Erickson G.F. 2015. *Morphology and Physiology of the Ovary.* MDText.com, Inc.; South Dartmouth, MA, USA: 2015.

27. Yang M., Lin L., Sha C., Li T., Zhao D., Wei H., Chen Q., Liu, Y., Chen, X., Xu, W., et al. 2020. Bone marrow mesenchymal stem cell-derived exosomal miR-144-5p improves rat ovarian function after chemotherapy-induced ovarian failure by targeting PTEN. Laboratory Investigation, 100, 342–352.

28. Zhang B., Zhang J., Shi H., Mao F., Wang J., Yan Y., Zhang X., Qian H., Xu W. 2018. A novel method to isolate mesenchymal stem cells from mouse umbilical cord. Molecular Medicine Reports, 17, 861-869.

29. Zhang X., Zhang L., Li Y., Yin Z., Feng Y., Ji Y. 2021. Human umbilical cord mesenchymal stem cells ,hUCMSCs promotes the recovery of ovarian function in a rat model of premature ovarian failure ,POF , Gynecological Endocrinology, 37 ,4 , 353-357.

30. Zhao Y.X., Chen S.R., Su P.P., Huang F.H., Shi Y.C., Shi Q.Y., Lin S. 2019. Using Mesenchymal Stem Cells to Treat Female Infertility: An Update on Female Reproductive Diseases. Stem Cells Int., 6, 2019:9071720.