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Stem Cells Transplantation in Premature Ovarian Failure

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Abstract: Stem cells (SC) are the foundation cells for every organ, tissue and cell in the body. They are undifferentiated "blank" cells that do not yet have a specific function. Under proper conditions, they begin to develop into specialized tissue and organs. They are self-sustaining and can replicate themselves for long periods of time. They have the remarkable potential to develop into many different cell types in the body. They serves as a sort of repair system for the body, they can theoretically divide without limit to replenish other cells as long as the person or animal is still alive. Premature ovarian failure (POF) is the loss of ovarian function in women less than 40 years. It is associated with sex steroid deficiency, amenorrhea, infertility and elevated serum gonadotropins. POF occurs in 1 % of women. In majority of cases the underlying cause is not identified. Management essentially involves hormone replacement and infertility treatment. This work aimed to evaluate the therapeutic potential of Autologous MSC transplantation in women suffering from Premature Ovarian Failure. Ten properly selected patients scheduled for Mesenchymal stem cells (MSC) transplantation at Al-Azhar University Hospitals. Mesenchymal stem cells (MSC) preparation from the bone marrow of the iliac crest was injected in the ovaries by laparscopy. Endometrial fractional biopsy is Immunohistochemically (IH) stained and evaluated according to Edessy stem cells score (ESS). The results indicated that after transplantation one case resumed menstruation after 3 months and 2 cases (20%) showed focal secretory changes after being atrophic endometrium. These cases were of ESS 5 and 6. It could be concluded stem cell transplantation is a promising procedure for cases of POF regarding the clinical, histopathological (HP) and IH outcome. The ESS is a suitable score for evaluating stem cell expression.

Key words: Autologous MSC • ESS • POF

INTRODUCTION

Stem cells are the foundation cells for every organ, tissue and cell in the body. Stem cells are undifferentiated "blank" cells that do not yet have a specific function [1]. Under proper conditions, stem cells begin to develop into specialized tissue and organs. Additionally, stem cells are self-sustaining and can replicate themselves for long periods of time [2]. Stem cells have the remarkable potential to develop into many different cell types in the body. Serving as a sort of repair system for the body, they can theoretically divide without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, or a brain cell. Stem cells differ from other kinds of cells in the body. All stem cells-regardless of their source, have three general properties: they are capable of dividing and renewing themselves for long periods, they are unspecialized and they can give rise to specialized cell types [3]. These unique characteristics make stem cell very promising for supplying cells to treat debilitating diseases like Alzheimer's disease, cancer, Parkinson's disease, type-1 diabetes, spinal cord injury, stroke, burns, heart disease, osteoarthritis, rheumatoid arthritis and end stage liver diseases.

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Today, donated organs and tissues are often used to replace those that are diseased or destroyed. Unfortunately, the number of people needing transplants far exceeds the number of organs available. Stem cells offer the potential for supplying cells and tissues, which can be used to treat these various diseases [4]. Different types of stem cells exist and within each type, there are many sources for the stem cells. There are many different protocols for separation, isolation and culture of stem cells to obtain lines of these cells for the potential therapeutic uses [3]. Premature ovarian failure (POF) is the loss of ovarian function in women less than 40 years. It is associated with sex steroid deficiency, amenorrhea, infertility and elevated serum gonadotropins [5]. POF occurs in 1 % of women [6]. In majority of cases the underlying cause is not identified [7].The known causes includes: (a) Genetic aberrations, which could involve the X chromosome or autosomes. A large number of genes have been screened as candidates for causing POF, however, few clear causal mutations have been identified, (b) Autoimmune ovarian damage, as suggested by the observed association of POF with other autoimmune disorders. Anti-ovarian antibodies are reported in POF by several studies, but their specificity and pathogenic role are questionable, (c) Iatrogenic following surgical, radiotherapeutic or chemotherapeutic interventions as in malignancies, (d) Environmental factors like viral infections and toxins for whom no clear mechanism is known [8]. The diagnosis is based on finding, of amenorrhea before age 40 associated with FSH levels in the menopausal range. Screening for associated autoimmune disorders and karyotyping, particularly in early onset disease, constitute part of the diagnostic work-up. There is no role of ovarian biopsy or ultrasound in making the diagnosis. Management essentially involves hormone replacement and infertility treatment [9]. Estrogen replacement is usually continued up to the age of 50 years, when the risk and benefit of continued treatment are reviewed [10]. This work aimed to evaluate the therapeutic potential of Autologous MSC transplantation in women suffering from POF.

MATERIALS AND METHODS

A prospective study comprised 10 patients scheduled for MSC transplantation at Al-Azhar University hospitals and Al Azhar Regenerative Medicine International Center (ARMIC). It was designed according to ethical committee rules of Al-Azhar University. For all women included in this study, explanation of the study procedures was done and informed consent was taken.

The Inclusion Criteria: Mesenchymal stem cell transplantation candidate, Post-menarche female less than 40 years old. Follicle-stimulating hormone (FSH) more than or equal to 20 IU/L, Female with normal karyotyping. Exclusion criteria: Breast cancer, Ovarian cancer, Female with abnormal karyotyping, Hypersensitivity to any Gonadotropin-releasing hormone (GnRH) analogs. They were subjected to Full Medical history, Clinical examination (General, abdominal and local examinations), Laboratory evaluation of the general condition by CBC, liver, kidney, thyroid tests and coagulation profile, Ovarian function evaluation (FSH, LH, E2, AMH), Gynaecological U/S (to assess the ovaries and the uterus)and Chromosomal study :to confirm normal karyotyping.

MSC Preparation: Sample of 10 ml is aspirated from the bone marrow of the iliac crest and prepared in the lab and injected in the ovaries through laparoscopy. Endometrial fractional biopsy was taken, stained with H&E stain and by IH staining by stem cell marker OCT4. Immunohistochemical expression of stem cell marker OCT4 was evaluated before and after transplantation according to ESS [11]. Participants were followed up monthly for a period of six months by hormonal (FSH, LH and E2), clinical (resuming menstruation), US (folliculometry), histopathological (HP) and IH expression of stem cell marker OCT4 of the endometrial biopsy (stem cell positivity according to ESS) outcome.

ESS:

	Score		
Factor	0	1	2
1- Intensity of sc marker.	Negative to mild	Moderate	Strong
2- Percentage of SC.	0	0-50%	>50-100%
3- Focality	None	Focal	Diffuse
4- Distribution	None	Epithelial or mesenchymal	Epithelial and mesenchymal
5- Site of SC	None	Cytoplasmic or nuclear	Cytoplasmic and nuclear

RESULTS

Evaluation of cases revealed that the age range of the patients was between 26-33 years. All of them were nullipara with normal karyotyping. Sixty per cent of the patients were in the middle social class and 70% were of urban residence. The relationship between the HP, IH and the ESS of the cases is shown in Table 1. It reveals that two cases (20%) developed FSE after being AE and DPE, both of them were with +v sc expression and with ESS of 5 and 6. Table 2 shows the clinical outcome of case in relation to the sc expression after transplantation. It reveals that 2cases developed +ve sc expression, out of them one case (50%) became menstruating with highly statistical difference (P<0.001). The Clinical outcome according to ESS is shown in Table 3. It reveals that 50% of cases with ESS=5 became menstruating after stem cell transplantation, while those with ESS<5 remained non menstruating and the difference was highly significant (P<0.001). Table 4 shows the HP evaluation in relation to the stem cell expression after t ransplantation. It reveals that the 2 cases with +ve sc expression developed FSE after being AE and DPE with highly statistical difference (p<0.001). Table 1 shows that HP evaluation revealed two cases (cases number 5 and 10) developed FSE changes after being DPE, while IH study with stem cell marker OCT4 revealed that these two cases (cases number 5 and 10) showed positive OCT4 IH staining after being negative before the transplantation. Both of them showed ESS of 6 and 5. Table 2 shows that after sc transplantation 2 cases developed +ve sc expression, out of them one case (50%) became menstruating with highly statistical difference (P<0.001). Table 3 shows that 50% of cases with ESS =5 became menstruating after stem cell transplantation while those with ESS<5 still non menstruating and the difference was highly significant (P< 0.001). Table 4 reveals that the 2 cases with +ve sc expression developed FSE after being AE and DPE with highly statistical difference (p<0.001).

Table 1: HP versus IH according to ESS.

	HP			ESS	
			IH		
Cases	Before	After	sc	Before	After
1	AE	AE	-ve	0/10	0/10
2	AE	AE showing low glands/ stromal ratio.	-ve	0/10	0/10
3	AE	AE showing low glands/ stromal ratio.	-ve	0/10	0/10
4	AE	AE	-ve	0/10	0/10
5	AE	DPE with FSE changes.	+ve	0/10	6/10
6	AE	PAE showing low glands/ stromal ratio with decidualized stroma.	-ve	0/10	0/10
7	AE	AE	-ve	0/10	0/10
8	AE	AE	-ve	0/10	0/10
9	AE	AE	-ve	0/10	0/10
10	DPE	DPE with FSE changes.	+ve	0/10	5/10

AE= Atrophic endometrium. DPE= Disordered proliferative endometrium. PAE= Pill Pattern atrophic endometrium. FSE= focal secretory endometrium.

Table 2: The clinical outcome in relation to the sc expression after transplantation.

	SC		
Return of menstruation	-ve.	+ve	P
Yes	0 /8 (0 %)	1/2 (50%)	<0.001
No	8/8 (100%)	1/2 (50%)	<0.001

Table 3: Clinical outcome according to ESS

	ESS		
	ESS ≤ 5	ESS ≥ 5	
Return of menstruation	No (8)	No (2)	P
Yes	0 (0%)	1(50%)	<0.001
No	8(100%)	1(50%)	<0.001

Table 4: HP versus stem cell expression after transplantation.

	SC		
Endometriumm	-ve SC=No. (8)	+ve SC = No. (2)	P
AE	8(80%)	0(0%)	<0.001
DPE with FSE.	0(0%)	2(40%)	<0.001

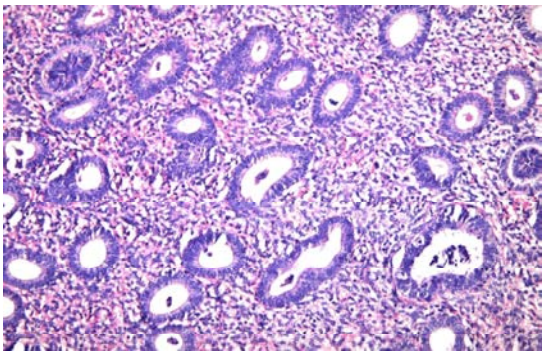

Fig. 1: DPE tissue with FSE changes showing rounded endometrial glands lined by single layer of cuboidal epithelium with basal nuclei. Some of the glands are dilated. The stroma is cellular with focal edema. (H & E stain x 200).

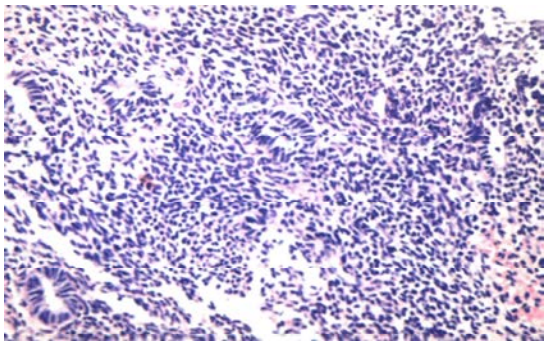

Fig. 2: PPE showing low glands/stromal ration with decidualized stroma(H &E stain x 200).

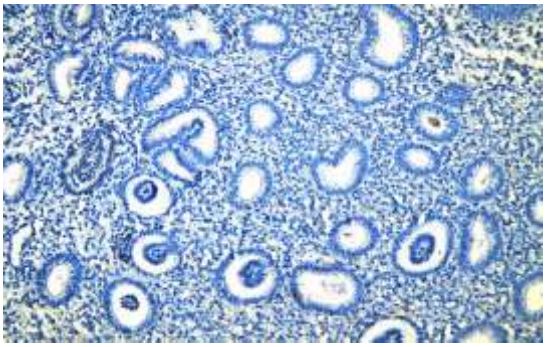

Fig. 3: Negative immunohistochemical staining of OCT4 stem cell marker of both glands and stroma(X100).

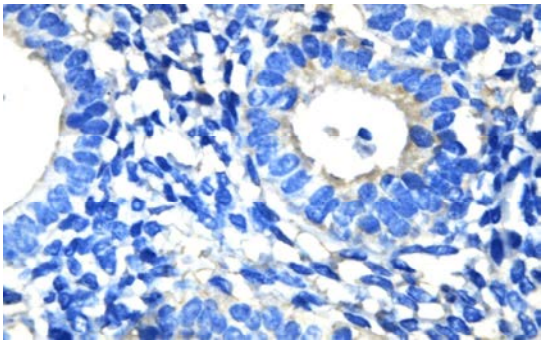

Fig. 4: Focal weak glandular immunohistochemical staining of OCT4 stem cell marker shown as focal glandular brown cytoplasmic staining and negative stroma. X 1000(oil immersion).

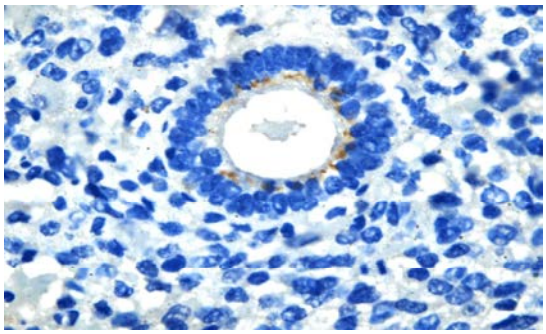

Fig. 5: Focal strong glandular immunohistochemical expression of OCT4 stem cell marker shown as brown cytoplasmic staining and negative stroma X1000 (oil immersion).

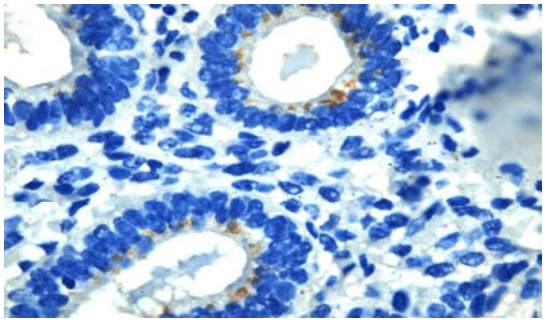

Fig. 6: Focal strong glandular immunohistochemical expression with OCT4 stem cell marker shown as brown cytoplasmic staining with negative stroma X1000 (oil immersion).

DISCUSSION

The current concept that the ovary has a static ovarian reserve is entirely at odds with the germ cell dynamics in its counterpart, the testis. The testis contains a sizeable number of germ stem cells and continuously

generates new gametes, even into old age. Although sperm numbers may diminish slightly at the extreme end of aging, recent research in mice suggested that this is mainly a result of aging of the niche rather than a defect in germ cells. The message that is given by Lee *at al*. [12] that the ovary is more like the testis, at least when exposed to chemotherapy. It is not a static organ and continues to produce new germ cells into adult life. Sixty mature female rats were studied. Fifteen rats as control group (Group I), 45 rats (the study group) were injected, by intra-peritoneal route, with cyclophosphamide (CTX) to induce ovarian failure. The study group was subdivided into 3 equal groups (Group II, III and IV). Male MSCs were injected intravenously into rats of Group II, while group III received PBS (Phosphate buffered saline) and group IV did not receive any injections. They were followed up for eight weeks by daily vaginal smear and biweekly serum estrogen (E2) and Follicle Stimulating Hormone (FSH). PCR was done to detect Sry gene expression. There was no statistical difference between group I and II as regards the mean FSH (3.60 ± 0.08 mIU/ml vs. 5.38 ± 0.31mIU/ml, P=0.1, respectively) and E2 levels (69.71±1.26 vs. 53.5±0.93 pg/ml, P=0.2, respectively) at 8 weeks. Cytological and histopathological examinations showed resurrection of ovarian folliculogenesis in group II only. The (Sry) gene expression was detected within the ovarian tissues in group II. According to the results, MSCs seem to have the power of recovering ovarian function both in its hormonal and follicular development abilities, in cyclophosphamide-induced ovarian damage in rats.

CONCLUSION

The Autologus mesenchymal stem cells transplantion showed promising results regarding the clinical outcome in the form of menstruation (10%) and the endometrial changes in the form of secretory changes (20%). Both the promising results showed +ve stem cell expression and ESS ≥ 5.

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