ORIGINAL ARTICLE

Autologous Stem Cells Therapy, The First Baby of Idiopathic Premature Ovarian Failure

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ABSTRACT

Introduction: Stem cells (sc) are the foundation cells for every organ, tissue and cell in the body. They are self sustaining and can replicate themselves for long periods of time. Stem cells can differentiate into different types of cells. Women below the age of forty and showing ovarian function loss are defined to have premature ovarian failure (POF). It is associated with sex steroid deficiency, amenorrhea, infertility, and elevated serum gonadotropins. **Aim:** To evaluate the therapeutic potential of autologous Mesenchymal sc (MSC) transplantation in women suffering from POF. Out of 112 high risk patients for POF(cases with amenorrhoea befor the age of forty), diagnosis was confirmed in10 cases. The ten POF patients were scheduled for MSC transplantation at Al- Azhar University Hospitals. MSC preparation from the bone marrow of the iliac crest was laparoscopically injected into the ovaries. Endometrial fractional biopsy was histopathologicaly (HP) and Immunohistochemically (IH) stained and evaluated according to Edessy stem cells score (ESS). Ovarian reserve was evaluated according to Edessy ovarian reserve score (EORS). **Results:** Showed that after transplantation two cases (20%) (ESS = 5 and 6) resumed menstruation after 3 months, one of them (10%) (Case no 5) (ESS = 6) got pregnancy after 11 months and delivered a healthy full term baby (Zeinab).Ten months after transplantation EORS of patient who developed pregnancy (case no 6) was found to be 7 after being 0 before therapy. EORS of the other menstruating case (case no 10) was 5 after being 0. The 2 menstruating cases showed focal secretory changes after being atrophic endometrium in case 5 and distorted proliferative endometrium in case 10. **Conclusion:** Stem cell transplantation is a good procedure and regarded as a real and hope to get healthy pregnancy and baby for cases of POF. It showed good clinical, HP and IH outcome.

INTRODUCTION

Edessy stem cells score (ESS) (Table 1)[1] is the objective method for the evaluation of the stem cell expression, while the Edessy ovarian reserve score (EORS) (Table2) [2] is the objective way for evaluating the ovarian reserve. Stem cells are self renewable undifferentiated cells capable of differentiation and divide into specialized cells.[3] Through the asymmetric mitosis the new cell differentiates into two daughter cells.[4] Adult stem cells, also called somatic stem cells, are stem which maintain and repair the tissue in which they are found. Both children and adults are sources for stem cells.[5] The umbilical cord and the adult tissue may contain the rare pluripotent stem cells in small amounts. End stage heart diseases, liver cirrhosis, spinal cord injuries and other conditions may be treated by stem cell transplantation using the adult bone marrow stem cells.[6] The quantity of bone marrow stem cells declines with age and is greater in males than

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females during reproductive years. Much adult stem cell research to date has aimed to characterize their potency and self-renewal capabilities.[7] Mesechymal stem cells are multipotent stem cells.[8] Leukemia has been treated since many years by stem cell transplantation. Destruction of the embryo makes the use of embryonic stem cells in research and treatment a matter of controversy which is not case for adult stem cells.[9] Amenorrhea and elevated gonadotrophins associated with loss of ovarian function in patients below forty years is the premature ovarian failure[10]According to Bretherick et al POF includes women below forty years and characterized by postmenopausal hypergonadotropism and hypoestrogenism.[11] POF occurs in 1% of the female population by the age 40, of whom 2.5% are adolescents, and is most often a non-reversible pathology leading to infertility. POF occurs in 1/10,000 at the age of 20 and 1/1000 at the age of 30 with normal karyotype. Primary amenorrhea with ovarian failure occurs in 1/10,000 women.[12] There is family history in about 25% of POF cases. Gonadal dysgenesis and Turner syndrome are the commonest causes of early POF which occurs below the age of 20 and the cause is unknown in POF cases with normal karyotyp and above 20 years.[13] 10–28% of primary amenorrhea cases and 4–18% of secondary amenorrhea cases are due to POF.[14] POF occurs in 0.1%, 0.5%, 1% and 1.4% of Japanese, Chinese, Caucasian and od African-American and Hispanic women respectively.[15] No etiology was accounted for about 90% of the idiopathic POF. Hormone replacement till the age of 50 was considered the main treatment.[16] Autologus mesenchymal stem cells transplantion in women with idiopathic or autoimmune premature ovarian failure restored ovarian functions in the form of restoration of menstruation (10%) and change from atrophic to secretory endometrium.[17] Stem cell transplantation showed promising procedure for cases of POF regarding clinical, histopathologicaly (HP) and Immunohistochemically (IH) outcomes.[18] This work aimed to evaluate the therapeutic potential of Autologous MSC transplantation in women suffering from POF.

MATERIALS AND METHODS

A prospective study was conducted at Al-Azhar University hospitals and Al Azhar Regenerative Medicine International Center (ARMIC). According to ethical committee rules of Al-Azhar University. 10 cases with POF were scheduled for laparoscopic ovarian autologus Mscs transplantation.

For all women included in this study, explanation of the study procedures was done and informed consent was taken. All cases were evaluated through medical history, general, abdominal and local examinations, laboratory tests (of the general condition by CBC, liver, kidney, thyroid tests and coagulation profile and ovarian function by FSH, LH, E2, AMH), gynecological, ultrasound and chromosomal study to confirm normal karyotyping.

The Inclusion Criteria

Mesenchymal stem cell transplantation candidate, Post-menarche females less than 40 years old. FSH more than or equal to 20 IU/L, normal karyotyping.

Exclusion Criteria

Breast cancer and ovarian cancer.

MSC Preparation

Sample of 10 ml was aspirated from the bone marrow of the iliac crest and prepared in the lab and injected into the ovaries through laparoscopy (Figures 1 and 2). Endometrial fractional biopsy was taken, stained with H&E stain and by IH staining by stem cell marker OCT4. Evaluation of the OCT4 was performed before and after transplantation according to Edessy stem cell score (ESS) (Table 1).

Follow Up

Participants were followed up monthly for a period of one year by hormonal (FSH, LH and E2), clinical (resuming menstruation and occurrence of pregnancy and its outcome), US (Ovary and uterus), HP and IH (stem cell positivity according to ESS) outcomes.

RESULTS

The patients mean age was 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 3. It reveals that two

Table 1: Edessy stem cell score (ESS)

Score factor	0	1	2
Intensity of SC marker	Negative to mild	Moderate	Strong
Percentage of SC	0	0-50	>50-100
Focality	None	Focal	Diffuse
Distribution	None	Epithelial or mesenchymal	Epithelial and mesenchymal
Site of SC	None	Cytoplasmic or nuclear	Cytoplasmic and nuclear

Edessy *et al.* 2014 (1)

Table 2: Edessy ovarian reserve score (EORS)

Score variable	0	1	2
AMH (ng/ml)	<1	1-5	>5
FSH (mlU/ml)	≥10	5-10	<5
E_2 (pg//ml)	<20	20-50	>50
AFC (no)	<3	3-9	>9
MOV (cm^3)	<6	6-10	>10

(FSH: Follicle stimulating hormone, E_2: Estradiol, AFC: Total antral follicle count and MOV: Mean ovarian volume.) Edessy *et al.* 2013[2]

cases (20%) (Case no 5 and case no 10) developed focal secretory endometrium after being atrophic endometrium and distorted proliferative endometrium (AE and DPE), both of them showed +ve sc expression after being –ve and with ESS of 6 and 5. Table 4 shows the clinical outcome of cases in relation to the sc expression after transplantation. It reveals that the 2 cases developed +ve sc expression and became menstruating, one of them get pregnant. The clinical outcome according to ESS is shown in Table 5. It reveals that 100% of cases with ESS≥5 became menstruating after transplantation (P<0.001), one of them got pregnant. Table 6 shows the HP evaluation in relation to the stem cell expression after transplantation. 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 7 shows the basic data of the pregnant case of the study while the hormonal profile of the pregnant case before and after transplantation is shown in Table 8.

Table 9 shows the HP, IH, ESS and EORS of the pregnant case before and after stem cell transplantation.

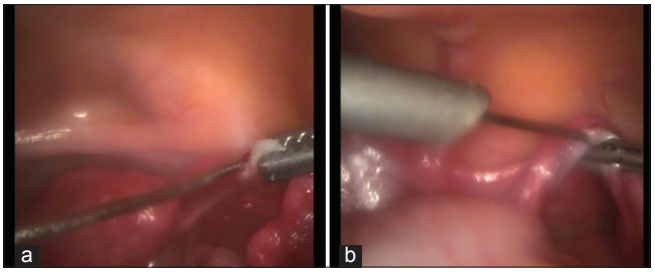

Figure 1: (a) Shows first step of laparoscopic ovarian autologous Mscs injection, (d) Shows second step of laparoscopic ovarian autologous Mscs injection

Table 3: HP versus IH according to ESS and EORS

Evaluation cases	HP		IH	ESS		EORS	
	Before	After	SC	Before	After	Before	After
1	AE	AE	–ve	0/10	0/10	0/10	0/10
2	AE	AE showing low glands/stromal ratio	–ve	0/10	0/10	0/10	0/10
3	AE	AE showing low glands/stromal ratio	–ve	0/10	0/10	0/10	0/10
4	AE	AE	–ve	0/10	0/10	0/10	0/10
5	AE	DPE with FSE changes	+ve	0/10	6/10	0/10	7/10
6	AE	PAE showing low glands/ stromal ratio with decidualized stroma	–ve	0/10	0/10	0/10	0/10
7	AE	AE	–ve	0/10	0/10	0/10	0/10
8	AE	AE	–ve	0/10	0/10	0/10	0/10
9	AE	AE	–ve	0/10	0/10	0/10	0/10
10	DPE	DPE with FSE changes	+ve	0/10	5/10	0/10	5/10

AE: Atrophic endometrium. DPE: Disordered proliferative endometrium. PAE: Pill pattern atrophic endometrium. FSE: Focal secretory endometrium

Figure 3 shows the negative immunohistochemical staining of OCT4 stem cell marker of both glands and stroma (X100), while Figure 4 shows the focal strong glandular immunohistochemical expression with OCT4 stem cell marker shown as brown cytoplasmic staining with negative stroma X1000(oil immersion).

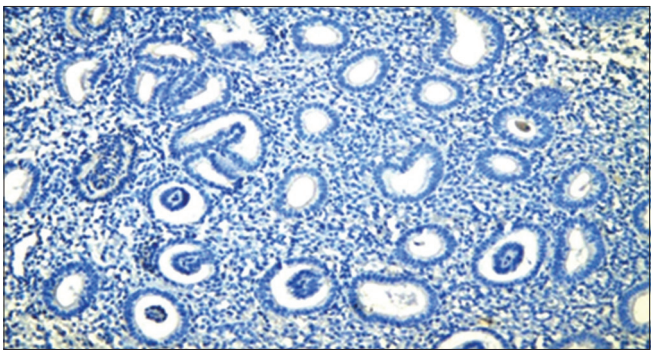

Figure 3: Negative immunohistochemical staining of OCT4 stem cell marker of both glands and stroma (X100).

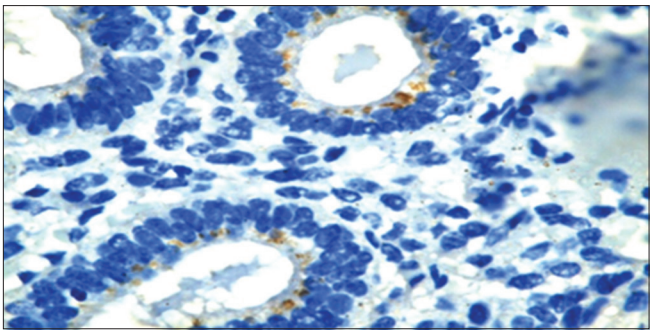

Figure 4: Focal strong glandular immunohistochemical expression with OCT4 stem cell marker shown as brown cytoplasmic staining with negative stroma X1000 (oil immersion)

Table 4: The clinical outcome in relation to the sc expression after transplantation

SC expression / Clinical outcome	–ve (8) (%)	+ve (2) (%)
Menstruation	0/8 (0)	2/2 (100)
Occurrence of pregnancy	0/8 (0)	1/2 (50)

Table 5: Clinical outcome according to ESS

ESS clinical outcome	>5 No (8) (%)	5 No (2) (%)
Return of menstruation	0 (0)	2 (100)
Occurrence of pregnancy	0 (0)	1 (50)

Table 6: HP versus stem cell expression after transplantation

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

Table 7: The basic data of the pregnant case of the study

Cases	Age	Parity	Menarche (years)	Age at 2ry amenorrhea	Duration of 2ry amenorrhea (years)	Karyotyping	Medical disorders	Exposure to radiation	Exposure to chemotherapy	Smoking	BMI
Case	30	NG	13	18	12	44+xx	No	No	No	No	31.2

Table 8: Hormonal profile of the pregnant case before and after transplantation (months)

Case	Before				After													
	Basal				1 month after injection			2 months after injection			6 months after injection				10 months after injection			
	FSH	LH	E2	AMH	FSH	LH	E2	FSH	LH	E2	FSH	LH	E2	AMH	FSH	LH	E2	AMH
Case	58	31	11	<0.1	120	35	50	97	65	13	64	35	20	3	13	17	150	5

Table 9: HP, IH, ESS and EORS of the pregnant case

Transplantation evaluation	Before transplantation	After transplantation
HP	DPE	DPE with FSE.
IH	−ve	+ve (Focal strong positivity of endometrial glands)
ESS	0/10	6/10
EORS	0/10	7/10

Stem cell transplantation not only resulted in improvement of the hormonal profile of the POF patient but also result in regaining menstruation and getting pregnancy and delivery of mature living healthy baby (Zeinab), 38 weeks pregnancy and 3.3 kg weight (Figure 5).

AE= Atrophic endometrium. DPE= Disordered proliferative endometrium. PAE= Pill pattern atrophic endometrium. FSE= Focal secretory endometrium

DISCUSSION

According to the previous experimental results in rats, ovarian function damage induced by cyclophsphamid was improved by MSCs therapy (follicular development and hormonal patterns).[19] That experimental study is confirmed by our study in the hormonal point of view where stem cell transplantation resulted in improvement of the hormonal profile from the ovarian failure state to the functioning ovary state. Our study confirmed the value of ESS as an objective method for the evaluation of the stem cell expression, and that the cut off point of ESS for menstruation at or above which menstruation occurs is 5 (the results which agrees with that of Edessy et al at 2014.[20] Also the cut off point of ESS for pregnancy is 6. The present study showed that stem cell transplantation is not only regained ovarian function but also regained menstruation (the result which agreed with that of Edessy et al at 2014[21] and was the cause for getting pregnancy.

The first baby of autologous stem cell therapy in POF is a real and hope.

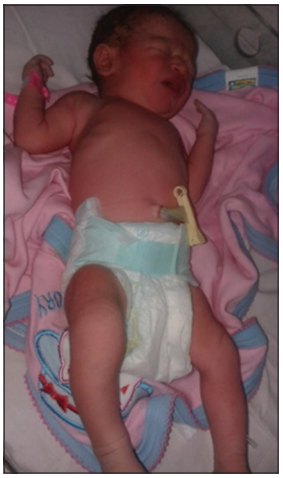

Figure 5: The first stem cell baby "Zeinab", mature living female, 38 wks, 3.3 kg

CONCLUSION

Stem cell transplantation is a good procedure and regarded as hope to get healthy pregnancy and baby for cases of POF. It showed good clinical, HP and IH outcomes. Further researches may be needed.

REFERENCES

1. Edessy, M., Hala N. Hosni, Olama.M. Sattar M and Monir M, 2014. Edessy Stem Cell Score (ESS), Endometrium, Endometrioma, Clinical Trials.gov Identifier: NCT02103452.,
2- Edssy M, Ali AEN, Fata A, Hamed W, 2013. Edessy Ovarian Reserve Score For Prediction of Assisted Reproduction Therapy Outcome. RCOG,678.
3. Edessy, M., Hala N. Hosni, Y. Wafa, S. Bakry, Y. Shady and M. Kamel,2014. Stem Cells Transplantation in Premature Ovarian Failure. RCOG 1620
4. Zhong W, Chia W,2008: Neurogenesis and asymmetric cell division. Curr Opin Neurobiol, 18(1):4-11.
5. "Stem Cells" Mayo Clinic,2013. Mayo foundation for medical education and research n.d Web. March 23.,
6. William JB, Prabakaran R, Ayyappan S, Puskhinraj H, Rao D, Manjunath SR, Thamaraikannan P, Dedeepiya VD, Kuroda S, Yoshioka H, Mori Y, Preethy SK, Abraham SJK (2011). "Functional Recovery of Spinal Cord Injury Following Application of Intralesional Bone Marrow Mononuclear Cells Embedded in Polymer Scaffold – Two Year Follow-up in a Canine". Journal of Stem Cell Research & Therapy 1 (3).

7. Dedeepiya VD, Rao YY, Jayakrishnan GA, Parthiban JK, Baskar S, Manjunath SR, Senthilkumar R, Abraham SJ (2012). "Index of CD34+ Cells and Mononuclear Cells in the Bone Marrow of Spinal Cord Injury Patients of Different Age Groups: A Comparative Analysis". Bone Marrow Res: 787414.
8. Gimble JM, Katz AJ, Bunnell BA (2007). "Adipose-derived stem cells for regenerative medicine". Circ Res 100 (9): 1249–60.
9. Stem Cell FAQ". US Department of Health and Human Services. 2004.07-14.
10. Edessy,M., Hala N. Hosni, Y. Wafa, S. Bakry, Y. Shady and M. Kamel,2014., Autologous Mesenchymal Stem Cells Transplantation In Women With Premature Ovarian Failure, ClinicalTrials.gov Identifier: NCT02062931.
11. Bretherick KL, Hanna CW, Currie LM, Fluker MR, Hammond GL, Robinson WP,2008. Estrogen receptor a gene polymorphisms are associated with idiopathic POF. Fertil Steril;89:318–324.
12. Panay N, Fenton A,2008. Premature ovarian failure: a growing concern. Climacteric; 11:1–3.
13. Kalu E, Panay N,2008. Spontaneous POF: management challenges. Gynecol Endocrinol;24:273–279.
14. Mamas L, Mamas E,2009. POF and dehydroepiandrosterone. Fertil Steril;91:644 646.
15. Cameron M, Grover S, Moore P, Jayasinghe Y,2008.Non-chromosomal, non-iatrogenic POF in an adolescent population: a case series. J Pediatr Adolesc Gynecol;21:38.
16. Hui ES, Udofa EA, Soto J, Vanderhoof VH, Zachman K, Tong ZB, 2006. Investigation of the human stem cell factor KIT ligand gene, KITLG, in women with 46, XX spontaneous POF. Fertil Steril;85:1502–1507.
17. Edessy M., Hala N. Hosni, Y. Shady, S. Bakr, and M. Kamel,2013. Autologous Stem Cells Transplantation in Premature Ovarian Failure. MSc thesis Al Azhar faculty of medicine (Assiut).
18. Edessy M., Hala N. Hosni, Y. Wafa, S. Bakry, Y. Shady and M. Kamel,2014. Stem Cells Transplantation in Premature Ovarian Failure. World Journal of Medical Sciences 10 (1): 12-16, ISSN 1817-3055.
19. Lee, H-J., K. Selesniemi, Y. Niikura, T. Niikura, R. Klein, D.M. Dombkowski and J.L. Tilly.,(2007).Bone marrow transplantation generates immature oocytes and rescues long-term fertility in a preclinical mouse model of chemotherapy-induced premature ovarian failure. J. Clin. Oncol., 25:3198–3204.
20. Edessy, M., Hala N. Hosni, Olama A.M. Sattar and A. Monir,2014. Edessy Stem Cell Score (ESS) Endometrium-endometrioma. World Journal of Medical Sciences 11 (1): 09-13, ISSN 1817-3055.
21. Edessy,M., Hala N. Hosni, Y. Wafa, S. Bakry, Y. Shady and M. Kamel, 2014., Pregnancy After Stem Cell Transplantation in Premature Ovarian Failure (POF), ClinicalTrials.gov Identifier: NCT02151890.

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