Treatment of Premature Ovarian Failure by Ultrasound Intra Ovarian Injection of Autologus Eye Tears

Ali Farid Mohamed Ali[1], Laila Farid[2], Yasmin Ali[3], Mohamed Ali[4]

Abstract: Introduction: Idiopathic premature ovarian failure is defined as cessation of menstruation before the expected age of menopause (40 years) without detectable cause. The definitive line of treatment is egg donation which is not accepted in many countries. Eye Tears is a miracle it has a unique composition so the aim of this work is to use ultrasound guided intra ovarian injection of Eye Tears as a new modality for treatment of idiopathic premature ovarian failure.**Material and method:**Three patients complaining of premature ovarian failure were enrolled in the study. Mean FSH = 121.6 ± 4.7 i.u/ml, Mean LH = 44.6 ± 8.9 i.u/ml,Mean E2 = 13.6 ± 3.4 pg/ml with nearly undetectable AMH. Eye Tears 0.2 ml was injected through Transvaginal ultrasound in both ovaries. Primary outcome is occurrence of menstruation and secondary outcome ovulation and pregnancy. Follow up twice monthly for FSH, LH, E2, progesterone and AMH menstruation, ovulation, and occurrence of pregnancy.**Results:**Statistically significant increase of E2, progesterone, AMH, VEGFand Micro RNA 126 and statistically significant decrease in FSH and LH. Menstruation (and ovulation) occurred in the first case after 2 months, in the second case it occurred after 4 months and in the third case it occurred after 3 months. In all cases pregnancy occurred after six months from the beginning of the treatment.**Conclusion:**Ultrasound intraovarian injection of Eye Tears is a new modality of infertility treatment of Idiopathic primary ovarian failure with no reported side effect.

Keywords: Eye Tears, premature ovarian failure, AMH, ovulation, menstruation

1 Introduction

Primary ovarian insufficiency (POI) or premature ovarian failure (POF) or premature menopause is defined as cessation of menstruation before the expected age of Menopause (40 years) clinically it is represented by amenorrhea, hypergonadotropic, hypoestrogenism (FSH level > 40iu/ml) although frequently stated that 1% of population is affected with (POF) the incidence of POF has increased in a recent years. The causes of this condition are mainly cytogenetic, genetic, infectious, iatrogenic, autoimmune, metabolic, and idiopathic [1, 2].The treatment should be directed to the cause, in the literatures the suggestedtreatment range from different protocols for ovarian stimulation [3], estrogen, alternative protocols involving cotreatment with andorgan, aromatase inhibitor, growth hormone Dehydroepiandrosteronesulfate [4], and egg donation [5].

Eye Tear is a miracle it contains many biochemicalsubstances which are; almost all types of electrolytes, specific small-molecules, proteins(protective/anti-infective), immune system and inflammatory mediators, neuropeptides, proteases and protease inhibitors, It also contains brain derived neurotrophic factor, aquaporins 3&9 and so many other chemical components[6]. So the aim of our work is to use Eye Tears in the treatment of premature ovarian failure.

1- *Ali Farid Mohamed Ali: Prof. and Ex-Chairman of the Department of Obstetrics and Gynaecology Ein-Shams University. Head of Heliopolis Infertility Research Center, Ex-Member of the Committee for Election of Prof. Degree in Obstetrics and Gynaecology – Cairo – Egypt. E-mail: ELShayb1950@yahoo.com*
2- *Laila Farid: Lecturer of Obstetrics and Gynaecology Ein-Shams University – Cairo – Egypt. E-mail: Lailamostafaf@gmail.com*
3- *Yasmin Ali: Lecture Pediatric and Intensive Care Ein-Shams University – Cairo – Egypt. E-mail: Elshayb1950@gmail.com*
4- *Mohamed Ali: Assistant Researcher, National Research Center – Cairo – Egypt. E-mail: mohamed_ali_farid@hotmail.com*

2 Material and method

Three cases were enrolled in the study mean age 30 ± 4 yearscomplaining of premature ovarian failure. Patients were subjected to the traditional lines of treatment which ranks from different protocols for ovarian stimulation [3], Estrogen, combined Estrogen and progesterone, alternative protocols involving treatment with andorgan, aromatase inhibitor, growth hormone and Dehydroepiandrosterone sulfate [4] but no response after many trials. Demographic, hormonal and vascularprofiles are illustrated in table 1&2

TABLE (1): HORMONAL PROFILE OF THE PATIENTS BEFORE ULTRASOUND INTRA OVARIAN INJECTION OF AUTOLOGUS EYE TEARS

No.	Age	FSH i.u/ml	LH i.u/ml	E2 pg./ml	Progesterone ng/ml	AMH
1	38	115	55	17	<0.01	<0.001
2	37	120	44	16	<0.001	<0.001
3	30	130	35	8	<0.01	<0.001

TABLE (2): VASCULAR PROFILE OF THE PATIENTS BEFORE

ULTRASOUND INTRA OVARIAN INJECTION OF AUTOLOGUS EYE TEARS

no	Vascular endothelial growth factor (VEGF)	Micro RNA 126
1	5.3	2.1
2	4.7	2.5
3	5.2	2.3

Regarding tears collection, we used the capillary tube for tears collection which is much less invasive and is suggested as the method of choice for biochemical studies. Tears samples were collected by placing a 2µl sterile microcapillary pipette (Drummond microcap) in the lower tear strip at the margin of external canthus of both eyes of each subject. (If stimulation of tears flow as a result of irritation by pipette was suspected, the sample was discarded [6, 7].

After full explanation to each patient, and her husband the procedure in detail a written consent was taken. The procedure was conducted under intravenous (IV) sedation and antibiotic cover, single dose of cefazolin (1000 mg) I.V. Patient was laid in Lithotomy position. Sterile disposable trans-vaginal probe and guide attached to it after locating both ovaries ovum pickup needle (Cook N 17) was introduced vaginally via the lateral fornix and the 0.2 ml of autologus Eye Tears was injected in the center of each ovary.Patients were discharged after 2 hours each, and antibiotic was given cefaxime 500 mg twice day for 3 days. Follow up for menstruation. FSH, LH, E2, progesterone, Antimullerin(AMH) hormone, Micro RNA 126, vascular endothelialgrowth factor and transvaginal ultrasoundto detectgrowing follicles and atretic follicles. The primary outcomeis occurrence of menstruation and secondary outcome wasOvulation and pregnancy.

3 Statistical Analysis

Statistical analysis was carried out by non-parametric using Mann Whitney U test.

To compare between FSH, LH, E2, progesterone,Antimullerin (AMH), Micro RNA 125, vascularendothelial growth factors, Number of growing and atretic,follicles before and after the procedure together with endometrial thickness before and after the procedure the threshold forsignificance was taken as $P< 0.05$.

4 Results

Follow up 2 weeks intervalrevealed statistically significant increase of E2, progesterone, AMH, VEGFand Micro RNA 126 ($P < 0.05$) and statistically significant decrease in FSH and LH ($P < 0.05$) Table (3),Table (4) and Table (5).3D Transvaginal ultra sound was used for detection of ovulation and endometrial thickness.Menstruation (and ovulation) occurred in the first case after 2 months, in the second case it occurred after 4 months and in the third case it occurred after 3 months. In all cases pregnancy occurred after six months from the beginning of the treatment. Ovulation was determined by transvaginal ultrasound and serum progesterone level. Table (6) showed statistically significant increase in the number of growing follicles and endometrial thickness together with decrease of the number of the atretic follicles than before the treatment. ($P< 0.01$)

TABLE (3): HORMONAL PROFILE OF THE PATIENTS AFTER ULTRASOUND INTRA OVARIAN INJECTION OF AUTOLOGUS EYE TEARS

No.	age	FSH i.u/ml	LH i.u/ml	E2 pg./ml	Progesterone ng/ml	AMH
1	38	9.7	25	95	8.7	1.7
2	37	8.7	29	87	9.5	1.5
3	30	10.1	20	92	8.9	1.9

TABLE (4): VASCULAR PROFILE OF THE PATIENTS AFTER ULTRASOUND INTRA OVARIAN INJECTION OF AUTOLOGUS EYE TEARS

no	Vascular endothelial factor (VEGF)	Micro RNA 126
1	21.2	8.4
2	22.8	12.5
3	26.9	11.5

TABLE (5): MEAN ± STANDARD DEVIATION OF FSH, LH AND E2 BEFORE AND AFTER ULTRASOUND INTRA OVARIAN INJECTION OF AUTOLOGUS EYE TEARS

	FSH i.u/ml	LH i.u/ml	E2 pg/ml
Before treatment	121.6 ± 4.7	44.6 ± 8.9	13.6 ± 3.4
After treatment	8.7 ± 2.6	24.6 ± 4.4	91.3 ± 3.7

TABLE (6): MEAN ± STANDARD DEVIATION OF ATRETIC, GROWING FOLLICLES AND ENDOMETRIAL THICKNESS BEFORE AND AFTER ULTRASOUND INTRA OVARIAN INJECTION OF AUTOLOGUS EYE TEARS

	Atretic follicles	Growing follicles	Endometrial thickness
Before treatment	6.8± 0.5	0	0.1 mm
After treatment	2 ± 1.3	9.1 ± 2.3	10.2 ±3.6 mm

$P < 0.01$ statistically highly significant

5 Discussion

Premature ovarian failure is defined as cessation of menstruation before age of 40 years the incidence of (POF) has increased in recent years. The treatment of this condition is directed to the cause [2]. But we confronted inthe majority of cases and our cases with no cause[1]many lines of treatment were suggested rank from different protocols for ovarian stimulation[3], estrogen, alternative protocols involving treatment with androgen, aromatase inhibitor, growth hormone, Dehydroepiandrosterone sulfate[4]and egg donation[5]with no reliable result. Due to the peculiar biochemical characters of the Eye Tears we used them in the treatment of premature ovarian failure to the best of our knowledge no report in the world literature dealt with this issue.

There are three types of Eye Tears; basal, reflex (from irritation), and emotional tears (physical tear) in humans. Tear film has three distinct layers (lipid layer, aqueous, mucus layer, watery layer sandwiched between an inner of lipids and outer fat [8].There are two methods to obtain Eye Tears capillary tubes [7] and Schirmer strips[9].Schirmer strips of tears collection is well established for measuring tear volume, however, the volume of tears collected by Schirmer strips is very small and obtaining an accurate composition for analysis is difficult. Evaporation of water from the small tear sample captured on the strip may significantly increase the apparent concentration of solutes, including antioxidant. Schirmer strips are also invasive and damage to occular surface cells by this strip could occur. It has been reported [10]that the use of Schirmer strips is associated with elevated plasmin concentration in the tear samples due to vascular fragility caused by strip induced irritation. For this reason we do not use this method to obtain Eye Tears.

Eye Tears is a miracle it contains almost all types of electrolytes (Sodium, Potassium, Calcium, Magnesium, Chloride, Bicarbonate, Nitrate, Phosphate, Sulfate), Small-molecule with good biological values(Retinol, VitaminC, Tyrosine, Glutathione, Glucose, Prostaglandin), Protein-Protective/anti-infective-(Lactoferrin, Lysozyme/muramidase, Phospholipase, Ceruloplasmin, CuZn superoxide dismutase, Lysosomal enzymes), Immune system/inflammatory (sIgA Secretory component, sIgM 5.6 μg/mL ,Complement components IL-1α, IL-1β,IL-Ra (IL-1 receptor antagonist), IL-2, IL-5 , IL-6,FasL, Tumor necrosis factor-α (TNF-α), Interferon-γ), Tear film maintenance (Lipocalin [tear – specific prealbumin (TSPA)], Albumin, MUC1 MUC4 (Sialomucin) MUC5AC ,MUC7),tears growth factors (Fibronectin, Gm-CSF protein, Transforming growth factor α (TGF-α), Transforming growth factor β 1 (TGF-β 1), Transforming growth factor β2 (TGF-β2), Tear hepatocyte growth factor (HGF), Keratocyte growth factor, Basic fibroblast growth factor (FGFβ; FGF2), Epidermal growth factor (EGF), Platelet-derived growth, factor BB, Insulin, Tenascin), Neuropeptides (Substance P, Calcitonin gene-related peptide), Proteases/ protease inhibitors (Plasminogen activator (urokinase type), Plasminogen/ plasmin, α 1-Antichymotrypsin, α 1-Protease inhibitor, α 2-Macrogloulin, Cystatins, Secretory leukocyte protease inhibitor (SLPI), Matrix metalloproteinase 2 (MMP-2), Matrix metalloproteinase 3 (MMP-3), Collagenase-2 (MMP-8), Matrix metalloproteinase 9 (MMP-9), Tryptase), it contains brain derived neurotrophic factor, aquaporins 3&9 , connexin 26 ,stem cells [6, 9] and endogenous very small embryonic like stem cells which is a very small embryonic like stem cells it exist in low numbers remain dormant under hemostatic conditions, it serves as back-up pool for adult stem cells and are mobilized under stress conditions, it is present also in adults gonad (ovary and tests) they survive oncotherapy it has the ability to undergo neo-oogenesis in the presence of healthy niche [11].

Tears are rich in anti-oxidants reactive oxygen species (ROS) involved in the process of ovulations.In emotional tears the increase in prolactin, ACTH, leucine, encephalin which cause stimulation of Dehydroepiandrosterone (DHEA) sulfate and Brain-derived neurotrophic factor (BDNF) which in turn stimulate the process of ovulation.

The protein content of tears provoked inflammatory reaction in the ovary, tear is rich in IL1 which is a key mediator of the inflammatory and immunological response, again tear prostaglandin help in the process of ovulation. In menopausal women it was suggested the potential presence of a female germ line stem cell population which provide a continual supply of primordialFollicle[12], mitotically active oogonial stem cells can be purified from adult mouse overies and human cortical tissue these cells can be propagated in vitro as well as generate oocytes in vitro and vivo [13]. Recently it was demonstrated the presence of endogenous very small embryonic like stem cells which serves as back-up pool for adult stem cells and are mobilized under stress conditions[11], It seems that eye tear by its molecular composition and by its content of stem cells stimulate these cells to differentiate to primordial follicles.

It was suggested that supply of appropriate blood vessels and the maintenance of vascular permeability in the ovaries and to follicles are necessary for gonadotropins to have an adequate effect and for paracrine factor to sustain follicular growth and ovulation [14, 15]. Vascular endothelial growth factor (VEGF) has the most potent angiogenic activity and vascular permeability activity, so it

participates in the regulation of early follicular growth[16]. Again, Vascular Endothelial Growth Factor (VEGF) stimulates the production of Nitric oxide which is Known to be a potent vasodilator and angiogenic factor plays a role in ovarian angiogenesis and ovulation) [17, 18]. It was reported that reproductive aged women may possess rare mitotically active germ cell that can be propagated in vitro as well as generate oocytes in vitro and in vivo by Eye Tears[13]. Eye Tears by its stem cells and biochemical composition stimulate ovarian stem cells which express germ cell marker (SSEA4, oct4, NANOG) exist in the ovarian surface epithelium. Putative stem cells with an embryonic character isolated from the ovarian surface epithelium of women with no naturally present follicles and oocyte[19, 20]. So we are now in a position that Eye Tears stimulate these ovarian stem like cells, and stimulates these rare mitotically active germ cells to production of oocyte.

It has been suggested that insulin growth factor (IGF-1) plays a role in the reinitiation of folliculogenisis.Injection Eye Tears could be followed by a cascade of inflammatory factors among which insulin growth factor 1 (IGF1) [21]

Representatives of the white blood cell series constitute a major component for the ovarian stromal (Interstitial) compartment. Macrophages present in permanent, noncyclic number may influence ovarian functions through the secretion of regularly cytokines. During the adult ovarian cycle there is infiltration of white blood cells in a pattern characterized by increase members of mast cells [21]. So in our cases, we produced the same events as occurred in adult natural ovarian cycle, by injection of Eye Tears. Eye Tears stimulate lymphocyte production which in turn increased progesterone production.

The existence of different T helper cell activities has long been proposed to account for the divergence of hormonal and cellular immunity to various stimuli. (Alteration in THI: TH2 ration can cause autoimmune disorder in animal's[22]models, alteration in this ratio hasbeen demonstrated in aged mice, and it is known thatAdministration of specific cytokines could restore that Tcell imbalance. We have demonstrated that injection of eye tears in aged female mice[23, 24] could restore the T cellimbalance. EyeTears are very rich in the following cytokines (Complement components IL-1α 43 pg/mL, IL-1β 30 pg/mL ,IL-Ra (IL-1 receptor antagonist) 295 ng/mL, IL-2 38 pg/mL, IL-5 40 pg/mL , IL-6 42 pg/mL ; 4.5 pg/mg protein ,FasL 0.30 ng/mL, Tumor necrosis factor-α (TNF-α) 0.36-1.97 ng/mL, Interferon-γ 91 pg/mL)which take over to correct the T cell imbalanceand hence correction of the divergence ofhormonal and cellular immunity[15, 16] which ultimately leads occurrence of ovulation So in our work the positive impact of eye tears on ovulation comes from hormonal, angiogenesis, inflammation and immunological aspect.

6 Conclusion

A new modality of infertility treatment of premature ovarian failure was introduced this modality acts through assembly of primitive follicle to primary follicle. Hormonal, angiogenic, inflammatory and immunological mechanisms were suggested as the mechanism of this modality. With no reported side effect and with acceptable cost benefit ratio. But and this is a very big But, many cases should be treated in many different center before this line of treatment comes on the clinical ground.

7 Acknowledgment

We acknowledge all who participated in this work either for giving help in preparation of the work to see the light, and to our patient who agreed to the publication of the condition for scientific benefit.

8 References

[1] **James N. Anasti:** premature ovarian failure an update Fertile Steril 1998: 70: 1-15

[2] **Liu T, X 41 uang, JI- Zhomg et al:** transplantation of human Menstrual Blood stem cells to treat premature ovarian failure in Mouse model. Stem cells and Development 2014 no 23 N 13 P isu8– 1557.

[3] **Barri P.N: ovarian stimulation in the older patient**: proceeding of IFFS 20th world congress on fertility and sterility. 2010 P227-230.

[4] **Leonidas Mamas, Eudoxia Mamas:** Premature ovarian failure and dehydro epiondrosterone. Fertil Steril 2009, 91: 644-66.

[5] **Van Kasteren XM. Schoemaker J**.: Premature Ovarian Failure a systematic review on therapeutic intervention to restore ovarian function and achieve pregnancy. Human Reprod Update 1999-5: 483-92.

[6] **Ali Farid Mohammed Ali:** Treatment of repeated implantation failure by Autologus eye tears. Fertility Magazine • Volume 14 •P 32-35.

[7] **Callender, M, Morrison, PE:** A Quantitative study of human tear proteins before and after adaption to non-flexible contact lens: Am J. optomophysiol OPT; 1974, 51: 939-945.

[8] **Ohiashiy, Dogru M, Tsubota K:** Laboratory finding in tear fluid analysis; Clin Chim Acta; 2006, 364 (1) 17-28.

[9] **Stephen C. Pflugfel der, Roger W. Beuerman, Michael Estern. Marcel Dekka:** Dry Eye and ocular surface disorder; 2004, 43-49.

[10] **William H. Freg II, Ph.D. Demise Desota. John Son, B.H.,and Carrie Hoffman, B.S and John T. MS call, Ph.D.** "Effect of stimulus on the chemical composition of human tear. American Journal of ophthalmology; 1982, 92: 559-567.

[11] **Deepa Bhartiya Et Al :** Endogenous, Very Small Embryonic-Like Stem Cells: Critical Review Human reproduction update vol 32 number 1 page 41-67 (2017)

[12] **Johnson J, Canning J, Kancho T. Pr, Jk. Tilly JL**: Germline stem cells and follicular renewal in the postnatal mammalian ovary Nature 2004; 428: 145-50.

[13] **White YAR, Woods DC, Takaix, ishihara O, Sekih, Tillyil**.: Oocyte formation by mitotically active germ cells purified from overies of reproductive age women Nat. Med 2012, 18: 413-21.

[14] **Gordon JD. Shifren JI. Foulk RA. Taylor RN, Jaffe RB.:** Angiogenesis in the human female reproductive tract. Obstet Gynecol Survy 1995; 50: 688-97.

[15] **Fraser HM.:Regulation** of the ovarian follicular vasculature. Reprodu BiolEndocrinol 2006. 4: 18-26.

[16] **Geva E, Jaffe RB.:** Role of vascular endothelial growth factor in ovarian physiology and pathology. Fertile Steril 2000; 74: 429-38.

[17] **Van der Zee R. Murohara T. Juo Z Zollmann f, Passeri J. Lckutat c, et al.:** Vascular endothelial growth factor (VEGF) vascular permeability factor (VPF) augments nitric oxide release from quiescent rabbit and human vascular endothelium. Circulation 1997, 95: 1030 -7.

[18] **Ferrara N. Navis Smyth T.:** The biology of vascular endothelial growth factor. Endocr Rev 1997; 18-4-25).

[19] **Virant-Klun, Zech N, Rozman P, Vogler A, et al.:** putative stem cells with an embryonic character isolated from the ovarian surface epithelium of women with no naturally present follicles and oocyte. Differentiation 2008: 76: 483-56.

[20] **Yohel Hagashi, Mitinooris Saitou and Shinya Yamanka,:**Germline development from human pluripotent stem cells toward disease modeling of infertility. Fertil Steril 2012, 97: 1250-9.

[21] **Larsen PR. Kronenbeg HM. Melmed, S. Polomsky K.S:** Williams Textbook of endocrinology 10th ed. Philadelphia Saunders 2003: 637-8.

[22] **Emi, N., Kanzaki, H., and Yoshida, M. et al:** Lymphocytes stimulate progesterone production by cultured human luteal cells. Am. J. Obstet. Gynecol., (1991) 165, 1468-74.

[23] **Mosmann TR, Coffinem RL.:** TH1 and TH2 cells different patterns of lymphokine secretion lead to different functional properties (AnnuRev immuol 1989: 7: 145-73).

[24] **Miller S.G:** gonado tropic control of ovarian follicular growth and development (2001) Mol. Cell. Endocrinol. 179, 39-46.