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Treatment of Premature Ovarian Failure by Ultrasound Intra Ovarian Injection of Bee Venom

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***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. Bee venom is a miracle it has a unique composition so the aim of this work is to use ultrasound guided intra ovarian injection of Bee venom as a new modality for treatment of idiopathic premature ovarian failure.

Patient and Methods:

A 30 years old woman married for 10 years diagnosed as idiopathic premature ovarian failure and primary infertility (FSH 120 iu /ml, LH 42 iu/ml, E2 3 ng/ml, progesterone < 0.00l ng, Antimullerian hormone (AMH) < 0.00001 Bee venom in the form of APIS – injeels Ampoule 0.2 ml was injected through trans vaginal ultrasound in both ovaries. Primary outcome is occurrence of menstruation and secondary outcome ovulation and pregnancy. Follow up monthly for hormonal level, growing follicles, menstruation, ovulation, and occurrence of pregnancy

Results:

Statistically significant decrease in FSH, LH, P < 0.001 and statistically significant increase in E2, progesterone, AMH, micro RNA 125, vascular endothelial factor P < 0.001, menstruation and ovulation had occurred 3 months from beginning of treatment pregnancy occurred 5 months from the beginning of treatment which result in delivery of female fetus 2.94 kg at 37 weeks gestation.

Conclusion:

Ultrasound intraovarian injection of Bee venom is a new modality of infertility treatment of Idiopathic primary ovarian failure with no reported side effect.

***Keywords*-- Premature ovarian failure, infertility, Bee venom, Antimullerian hormone (AMH), ultrasound.**

I. 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 but many cases are idiopathic, in the literatures the suggested treatment range from different protocols for ovarian stimulation,[3]estrogen, alternative protocols involving cotreatment with andorgan, aromatase inhibitor, growth hormone dehydroepianderosterone sulfate[4], and egg donation[5].

Bee venom is a miracle, it has a unique composition, it contains at least 28 active components, enzymes, coenzymes, peptides and stimulation of gap junctional intercellular communication (GJIC) connexin 26. biogenic amines[6, 7] Bee venom induced ovulation[8] and more ovulation and pregnancy rate than Human menopausal gonadotropin[9].

So the aime of this work is to use ultrasoundguided intraovarian injection of Bee venom as a new modality for infertility treatment of premature ovarian failure.

II. Patients And Method

A 30 years old woman married for 10 years had prolonged period of infertility, and Idiopathetic premature ovarian failure E2 (Estradiol 3 Pg/ml progesterone < 0.001ng micro RNA 126 2.2 Vascular endothelial factor 5.3

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FSH 120 iu/ml, LH 42 iu/ml. Antimullerian hormone (AMH) < 0.00001 almost approaching to zero level, the patient was 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 dehydroepianderosterone sulfate[4] but no response through 20 years, due to religious and ethical causes the patient refused egg donation and accept our line of treatment[5].

After full explanation to her, and her husband a written consent was taken after counseling and explaining the procedure in detail. Bee venom was used in the form of 0.1 ml/ampoule (Apis – Injeels (Heel GmbH Baden Germany, the does injected in each ovary. Is based upon FSH level: 40-80 (one ampoule 0.1 ml., 80-120 (2ampoule) above 120iu/ml 3 ampoule. (Farid equation) [8].

The procedure was conducted under intravenous ($_{IV}$) sedation and antibiotic cover, single dose of cefazolin $_{IV}$). Patient was laid in Lithotomy position. sterile disposable transvagainal 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 Bee venom was injected in the center of each ovary according to Farid equation[8].

Patient was discharged after 2 hours and antibiotic was given cefaxime 250 mg twice day for 3 days. Follow up for menstruation. FSH, LH, E2, progesterone Antimullerin (AMH) hormone, Micro RNA 126, vascular endothelial growth factor, and transvaginal ultrasound. To detect growing follicles and atretic follicles. The primary outcome is occurrence of menstruation and secondary outcome was Ovulation and pregnancy.

III. Statistical Analysis

Statistical analysis was carried out by non parametric using Mann whitney *U* test[1].

To compare between FSH, LH, E2, progesterone Antimulleruian hormone (AMH), Micro NA 125 vascular endothelial growth factors, Number of growing and atretic, follicles before and after treatment, the threshold for significance was taken as $p < 0.05$.

IV. Results

Follow up monthly for FSH, LH, E2, progesterone AMH, RNA 125, VEGF, occurrence of menstruation, 3D trans vaginal ultra sound for detection of ovulation.

Menstruation (and ovulation) had occurred 3 months from the beginning of the treatment pregnancy occurred 5 months from the beginning of treatment.

Ovulation was determined by transvaginal ultrasound increased number of growing follicles 8 ± 2.1 versus Zero after treatment P<0.001, atretic follicles 2 ± 1.1 versus 6 before treatment P< 0.001 Endometrial thickness 0.1 ml versus 10 ± 2.7 after treatment P< 0.001

Regarding the hormonal profile before and after treatment revealed FSH 120 miu/ml versus 10 ± 1.8, LH 42 miu/ml versus 12 ± 1.7 E2 3 versus 120 ± 2.8 progesterone on day 21 of the cycle (0.001) versus 16.8 ± 3.55). < 0.0001 AMH

versus 1.9 ± 0.36 versus< 0.0001 after the treatment statistically highly significant P < 0.001. regarding the angiogenic markers (micro RNA 125, VEGF).

micro RNA125 before treatment 3.1 ± 1.8 versus 60.2 ± 3.4 (after treatment) VEGF 40 ± 2.2 versus 4.1 ± 1.1 after treatment P < 0.0001 statistically highly significant.

So all the angiogenic and ovulation markers are statistically significant increased. positive pregnancy test was detected after the fifth cycle from beginning of the treatment.

The course of pregnancy passed smooth with no medical or obstetrics complications, elective caesarean section was done at 37 weeks gestation, delivery of female foetus 2.94 kg Apgar score 1,5 minute was 8, 10 respectively the post netal period passed with no complications.

V. 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 (the majority of cases and our case 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, dehydroepianderosterone sulfate[4] and egg donation[5] with no reliable result. Regenerative medical researches suggest that due to self-renewal capacity of stem cells they could be used to treat various human diseases this till now was experimental issue[2], so in between the previously mentioned lines of treatment and experimental future line of treatment come our innovation, to the best of our knowledge no report in the world literature dealt with this issue.

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Bee venom is a miracle it contains at least 28 active component, many coenzymes and enzymes, peptides and biogenic amines[6,7] and stimulation of gap junctional intercellular communication (GJIC) connexin 26. Bee venom induce the mechanistic target of rapamycin complex (TORCI) activation path way and phosphatase and tensim homology deleted on chromosme 10 AKT (PTEN) inhibitor path way[5] in the ovarian follicle this will lead to primordial follicle activation[10,11]. Bee venom induced ovulation[8], improves ovarian responsiveness[6,12], increase ovulation and pregnancy rate more than human menopausal gonadotropin[9], increase pregnancy rate in poor responders, in this work menstruation had occurred three months from the beginning of the treatment, the pooled result of all hormonal profile (FSH, LH, E2, progesterone, AMH) (till pregnancy occurred After 3rd cycle of treatment) revealed statistically highly significant change after treatment $P<0.001$.

Ovulation was demonstrated on 3 D transvaginal ultrasound by demonstration of a growing follicles the pooled number of the treatment of period was 8 ± 2.1 statistically highly significant the diameter of the follicles range from 16-21 mm with mean of 17 mm. together with increased endometrial thickness pooled mean ± SD till the occurrence of pregnancy was 10.2 ± 1.6 statistically highly significant ($P < 0.001$).

Ovarian factors are the main problem of infertility in menopausal women these include: decreased number of oocyte[3], chromosomal abnormalities[13] and injury of mitochondrial[14]. The decreased number of oocyte result from loss of the mechanism for the transition from primordial follicle to primary follicle rather than the depletion of primordial follicle[15,16]. on the clinical ground pre menopausal patients tend to have low ovarian reserve (low Antimullerian hormone and high basal FSH, low antral follicle count with more than half of the oocytes are aneuploidies[17]. In menopausal women it was suggested the potential presence of a female germ line stem cell population which provide a continual supply of primordial follicle[18], 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[19].

In our case we demonstrated increase in miRNA 126 and vascular endothelial growth factors, $P<0.0001$ microRNAs (miRNAs) are 21 nucleotide (nt) single stranded noncoding RNAs that bind to target mRNA[20] is important for many aspect of development, homoeostasis, and disease. miRNA 126 is involved in the regulation of the angiogenic process and vascular intergrity[21].

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[22,23]. 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[24]. 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) [25,26]. 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[19]. Ovarian stem like cell 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[27,28]. So we are now in a position that bee venom 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) plyas a role in the reinitiation of folliculogenisis injection bee venom could be followed by a cascade of inflammatory factors among which insulin growth factor 1 (IGF_1) [9], [30].

Representatives of the white blood cell series constitute a major component for the ovarian stromal (Interstitial) compartment. Macrophages present in permanent, non-cyclie number may influence ovarian functions through the secretion of regularly eytokines. During the adult ovarian cycle there is infiltration of white blood cells in a pattern characterized by increase members of mast cells[30]. So in our case, we produced the same events as occurred in adult natural ovarian cycle. By injection of bee venom. Bee venom stimulate lymphocyte production[6,7] which in turn increased progesterone production[30].

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[31] models, alteration in this ratio has been demonstrated in aged mice, and it is known that Administration of specific cytokines could restore that T cell imbalance. We have demonstrated that injection of Bee venom in aged female mice[32,33] could restore the T cell imbalance and hence correction of the divergence of hormonal and cellular immunity[6,23,24].

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So in our work the positive impact of bee venom on ovulation comes from hormonal, angiogenesis inflammation and immunological aspect. (These together with no reported side effect and low cost of the procedure making this line of treatment to have a leading position when other methods of treatment failed or to use it from the start.

VI. Conclusion

A new modality of infertility treatment of premature menopausal women was introduced this modality acts through assembly of primitive follicle to primary follicle. Hormonal, angiogenic, inflammatory and immunological mechanisms was 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.

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