INTRODUCTION

Infertility refers to the failure to conception by the couple and is perceived as a multifactorial syndrome in all cultures and societies.1 Out of the offered treatment procedures in reproductive clinics, in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the advanced reproductive techniques (ART). In these procedures, ovaries are down-regulated and then stimulated to produce eggs, which retrieved, and then microinjected with spermatozoa.2,3 The success of the procedure depends on quality of embryos and endometrial receptivity offered at the time of implantation.4

One of the elementary steps for success depends on a number of eggs obtained at the end of controlled ovarian stimulation (COSt). Females show a different response to stimulation on the
The success of treatment procedures is influenced by factors such as ovarian responsiveness and gives an insight to the estimation of hormone levels on the day of human chorionic gonadotropin (hCG) that gives an indirect evidence of the presence of follicles. The estradiol (E2) produced by the granulosa cells of the ovaries upregulates progesterone receptors for preparation of blastocysts implantation in the luteal phase of a normal menstrual cycle. Luteal phase E2 stimulates progesterone receptors in the endometrial glands and stroma during follicular and early luteal phases of a normal menstrual cycle. Luteal phase E2 stimulates progesterone receptors and also proliferate endometrial gland resulting in hypertrophy and hyperplasia of endometrial epithelia which may or may not favor implantation.

Pituitary down-regulation for COS reduces E2 as well as P levels in the luteal phase. This decline of serum E2 and progesterone levels in mid-luteal phase of normal menstrual cycle can adversely affect the results of implantation and successful pregnancy during IVF/ICSI cycles and increase rate of non-conception and biochemical pregnancies.

In spite of a variety of protocols and supportive therapies, poor responders continue to represent a challenge to IVF experts. The addition of E2 in the luteal phase for improvement in the rate of pregnancy or implantation rates in the selected cases of poor responders is still a subject of debate. In this study, we wanted to assess mid-luteal estradiol (E2) levels in poor and good responders and compare its effect on the outcome of ICSI in our local population. The results of the study may help in identification of the patients (poor/good responders) who could benefit with the supplementation of E2 supplements.

METHODS

The current study was conducted from June 2011 to September 2013 after ethical approval from Institutional Review Board of “Islamabad Clinic Serving Infertile Couples” All clinical investigations followed principles of Declaration of Helsinki. Females included in the study had primary infertility for more than two years with the normal menstrual cycle of 25 ± 7 days. The Baseline investigations included; (day three follicle stimulating hormone; FSH) and antral follicle count (AFC) done by the transvaginal scan. All females with polycystic ovaries and anatomical or morphological abnormalities were excluded.

These patients had down-regulation with long protocol by use of injection decapetide, (Ferring, Copenhagen NV) and COS with recombinant FSH for a period of 12± two days. The ovulation induction (OI) was done by injection of 10,000 IU of human chorionic gonadotropin (HCG) after confirmation of maturity of at least two follicles acquiring a size of 18-20 mm. The venous sample was obtained for measurement of peak estradiol E2 on OI. Oocyte retrieval was performed on 35-hour after OI by the transvaginal route under ultrasound guidance followed by embryo transfer three days after the procedure. Mid-luteal estimation of E2 was done on the day of transfer and groups (A-E) were stratified based on percentile values 20th, 40th, 60th and 80th percentile with 841, 948, 997 and ≥1081.2 pg/ml E2 levels. Clinical pregnancy was demarcated by the existence of a gestational sac with cardiac activity observed by TVS at the 7th week of gestation.

Statistical Analysis: The data was analyzed on SPSS 21, and continuous variables were represented by mean and standard deviation. Student’s t test was employed for comparing two groups (poor/good responders) and p<0.05 considered significant.

RESULTS

Females enrolled in the study (842) were categorized into groups of poor (513) and good (329) respectively with an age range of 32.25±4.3 years. Conception occurred in 365 patients, 100 (27%) poor and 265(73%) good responders, respectively. The comparison of characteristics in overall, poor and good responders are given in Table-I. The difference in several variables between poor and good responders, like age of menarche, body mass index (BMI), peak estradiol (E2) levels on the day of transfer and groups (A-E) were stratified based on percentile values 20th, 40th, 60th and 80th percentile with 841, 948, 997 and ≥1081.2 pg/ml E2 levels. Clinical pregnancy was demarcated by the existence of a gestational sac with cardiac activity observed by TVS at the 7th week of gestation.
index (BMI), antral follicle count, estradiol before treatment, the length of stimulation, preovulatory follicle count, the number of fertilized oocyte cleaved and transferred embryos and endometrial thickness was significant. Table-II shows peak and mid luteal E2 in all patients, good responders, and poor responders. The peak E2, mid luteal E2 were significantly high in pregnant females in all three groups (p<0.05), while peak/mid-luteal E2 ratio was significantly lower in all three groups (p<0.05).

Fig.1 shows the comparison of clinical pregnancy rate in poor and good responders on the basis of mid E2 levels. Based on stratification in groups (A-E), Group A comprised of 123 poor and 45 good responders, all failed to conceive. In-group B, 10/60(17%) good responders conceived out of 168 females. The pregnancy rate was 9.5%(12/126), 66.7%(30/45) in poor and good responders of

| Variables                  | Overall (842) | Poor responders (513) | Good responders (329) | P value |
|----------------------------|---------------|-----------------------|-----------------------|---------|
| Duration of infertility    | 7.11 ± 3.9    | 6.97 ± 3.6            | 7.3 ± 4.2             | 0.22    |
| Female age                 | 32.11 ± 4.6   | 31.97 ± 4.6           | 32.3 ± 4.7            | 0.31    |
| Age of menarche            | 14.05±1.17    | 14.18±1.2             | 13.87±1.0             | 0.000   |
| Estradiol before treatment (pg/ml) | 214.7±145.6   | 190.36±130.6          | 246.70±157.9          | 0.000   |
| Antral Follicle Count      | 14.66±2.80    | 14.93±2.3             | 14.29±2.5             | 0.001   |
| BMI                        | 24.24 ± 3.7   | 24.51 ± 3.7           | 23.9 ± 3.7            | 0.01    |
| Length of stimulation      | 14.34 ± 1.0   | 14.44 ± 1.0           | 14.2 ± 1.0            | <0.001  |
| No. of oocytes/patient     | 7.69 ± 1.7    | 7.11 ± 1.7            | 8.45 ± 1.2            | <0.001  |
| No. of oocytes Metaphase II| 7.13 ± 2.0    | 6.2 ± 2.0             | 8.34 ± 1.1            | <0.001  |
| No. of oocytes fertilized  | 5.95 ± 1.6    | 5.18 ± 1.6            | 6.96 ± 0.7            | <0.001  |
| Endo. Lining               | 8.6 ± 3.4     | 7.81 ± 3.3            | 9.63 ± 3.3            | <0.001  |
| No. of transferred embryos | 1.62 ± 0.6    | 1.58 ± 0.6            | 1.68 ± 0.6            | 0.01    |

BMI=Body mass index, PFC= Preovulatory follicle count,  Endo= endometrial thickness in mm, No.= number.

| Variables                  | Pregnant                     | Not pregnant                | P value |
|----------------------------|------------------------------|-----------------------------|---------|
| Peak and mid luteal E2 in all patients | 2556.51 ± 173.1             | 2404.27±157.6               | <0.001  |
| Peak E2 (pg/ml)            | 1121.94 ±139.9              | 876.61 ± 98.8               | <0.001  |
| Mid luteal E2 (pg/ml)      | 2.30 ± 0.3                   | 2.77 ± 0.3                  | <0.001  |
| Peak and mid luteal E2 in good responders | 2556.507 ± 173.13            | 2404.272±157.5               | <0.001  |
| Peak E2 (pg/ml)            | 1121.943 ±139.8             | 876.61 ± 98.8               | <0.001  |
| Mid luteal E2 (pg/ml)      | 2.305 ± 0.25                 | 2.77 ± 0.3                  | <0.001  |
| Peak and mid luteal E2 in poor responders | 2440.99 ± 227.2             | 2143.01 ± 286.4             | <0.001  |
| Peak E2 (pg/ml)            | 1069.42 ±114.0              | 907.06 ± 128.0              | <0.001  |
| Mid luteal E2 (pg/ml)      | 2.29 ± 0.2                   | 2.40 ± 0.4                  | 0.04    |

E2= estradiol, Values are Mean ± SD.
Group C. Females (164) with mid-luteal levels ≥ 997 pg/ml in Group D had 38% (30/78) and 90% (78/86) pregnancy rate in poor and good responders respectively. In-group comprising of 171 females 123/126 good responders (98%) conceived in comparison to 21/45, 53% poor responders. The conception rate in females with mid-luteal E2 levels below 20th percentile was zero in both poor and good responders, but this rate gradually increased in with mid-luteal E2 levels above 40th, 60th and 80th percentiles, with maximum conception rate above 80th percentile indicating that high mid-luteal E2 levels indicate outcome of conception in ICSI especially in good responders.

**DISCUSSION**

The ART clinics try their level best to plan treatment plans with minimum complications in terms of selection of patients, a number of visits, type of protocol, injections for stimulation; their cost vs. side effects in comparison to patient’s satisfaction and clinical pregnancy rate. The measurement of peak and luteal E2 is done in these patients keeping in mind its importance for proliferation of endometrium and up-regulation of progesterone receptors required in IVF and ICSI cycles. 

Studies have shown that high peak and mid-luteal E2 can predict the success of treatment after ICSI by the provision of optimal environment required for implantation of fertilized ovum and accomplishment of clinical pregnancy. In our study, peak and mid luteal E2 levels were higher in pregnant females as compared to the non-pregnant group which is similar to other studies. 

Similarly, trends were seen in studies showing significantly high E2 and progesterone in females who have conceived and with on-going pregnancy as compared to non-conception group and females with miscarriages respectively, showing that both E2 and progesterone in mid-luteal phase can predict clinical pregnancy outcome in IVF/ICSI cycles. 

On the contrary, studies have established no role of mid-luteal E2 in the improvement of pregnancy. There are also contradictory randomized controlled trials, in which addition of E2 through oral medications in luteal phase did not improve IVF/ICSI outcomes.

In our study, peak and mid luteal E2 levels were significantly high in pregnant as compared to non-pregnant females in both poor and good responders, while peak to mid luteal estradiol ratio was low in pregnant as compared to non-pregnant females in both poor and good responders. Studies have also shown significantly high mid-luteal estradiol levels in pregnant females in good responders but no such significance was seen in poor responders.

The conception rate in females with mid-luteal E2 levels below 20th percentile was zero in both poor and good responders, but this rate gradually increased in good responders with mid-luteal E2 levels above 40th, 60th, and 80th percentiles, with maximum conception rate above 80th percentiles indicating that high mid-luteal E2 levels indicate outcome of conception in ICSI especially in good responders.

**CONCLUSION**

Maximum pregnancies in poor and good responders (53% and 98% respectively) occurred with mid-luteal E2 levels above 80th percentiles. The results confirm the role of the increase in mid-luteal E2 for augmentation in conception rate of females after ICSI. Further experimental trials are required to explore the usefulness of E2 supplementation for support of conception after ICSI.

**Declaration of interest:** The authors declare that they have no conflict of interest.

**REFERENCES**

1. Rehman R, Khan R, Baig M, Hussain M, Fatima SS. Estradiol progesterone ratio on ovulation induction day: a determinant of successful pregnancy outcome after intra cytoplasmic sperm injection. Iran J Reprod Med. 2014;12:633-640.
2. Rehman R, Hussain Z, Siddiq AA. Role of Progesterone in human embryo implantation. Rawal Med J. 2012:37:194-198.
3. Rehman R, Hussain Z, Zuberi NA. Prediction of success in intracytoplasmic sperm injection (ICSI) by estimation of serum Estradiol/Progesterone ratio on the day of embryo transfer. J Pak Med Assoc. 2013;63:609-613.
4. Aktan E, Bozkurt K, Ozer D, Yucebilgin S, Karadadas N, Bilgin O. The effect of mid-luteal estradiol level on the outcome of ICSI-ET cycles. Arch Gynecol Obstet. 2004;269:134-138. doi:10.1007/s00404-003-0353-6
5. Ubaldi F, Vaiarelli A, D’Anna R, Rienzi L. Management of Poor Responders in IVF: Is there anything new? Bio Med Res Int. 2014(2014), Article ID 352098. doi: 10.1155/2014/352098.
6. Ferraretti AP, La-Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L.ESHRE consensus on the definition of “poor response” to ovarian stimulation for in vitro fertilization: the Bologna criteria. Human Reprod. 2011;26:1616–1624. doi: 10.1093/humrep/der92.
7. Rehman R, Jawaid S, Gul H, Khan R. Impact of peak estradiol levels on reproductive outcome of intracytoplasmic sperm injection. Pak J Med Sci. 2014;30(5):996-991. doi: 10.12669/pjms.305.5175.
8. Rehman R, Hussain Z, Zahir H, Khan R. Impact of peak mid luteal estradiol on pregnancy outcome after intracytoplasmic sperm injection. J Pak Med Assoc. 2014;64:780-784.
9. Drakakis P, Loutradis D, Vomvolaki E, Stefanidis K, Kiapkekou E, Anagnostou E, et al. Gynecol Endocrinol. 2007;23:645-652. doi:10.1080/09513590701664923.
10. Engmann L, DiLuigi A, Schmidt D, Benadiva C, Maier D, Nulsen J. The effect of luteal phase vaginal estradiol supplementation on the success of in vitro fertilization treatment: a prospective randomized study. Fertil Steril. 2008;89:554-561. doi:10.1016/j.fertnstert.2007.04.006.

11. Meena DK, Mohammed IT, Vijaya T, Zakiya A. Changes in cytokines, biomarkers of bone turnover and hormones are associated with bone loss in postmenopausal Indian women. Int J Endocrinol Metab. 2012;1:399-403.

12. Oehninger S. Poor responders in in vitro fertilization (IVF) therapy: the challenge continues. Facts Views Vis. Obgyn. 2011;3(2):101-108.

13. Serna J, Cholquevilque JL, Cela V, Martínez-Salazar J, Requena A, García-Velasco JA, et al. Estradiol supplementation during the luteal phase of IVF-ICSI patients: a randomized, controlled trial. Fertil Steril. 2008;90:2190-2195. doi:10.1016/j.fertnstert.2007.10.021

14. Groothuis PG, Dassen HH, Romano A, Punyadeera C. Estrogen and the endometrium: lessons learned from gene expression profiling in rodents and human. Hum Reprod Update. 2007;13:405-417. doi:10.1093/humupd/dnm009

15. Gelety TJ, Buyalos RP. The influence of supraphysiologic estradiol levels on human nidation. J Assist Reprod Genet. 1995;12:406-412. doi:10.1007/BF02211139

16. Sonntag B, Loebbecke KC, Nofer JR, Kiesel L, Robert RG. Serum estradiol and progesterone in the mid-luteal phase predict clinical pregnancy outcome in IVF/ICSI cycles. Gynecol Endocrinol. 2013;29:700-703. doi:10.3109/09513590.2013.797392

17. Sharara FI, McClamrock HD. Ratio of oestradiol concentration on the day of human chorionic gonadotrophin administration to mid-luteal oestradiol concentration is predictive of in-vitro fertilization outcome. Human Reprod. 1999;14:2777-2782. doi: 10.1093/humrep/14.11.2777.

18. Ng EHY, Yeung WSB, Lau EYL, So WWK, Ho PC. A rapid decline in serum oestradiol concentrations around the mid-luteal phase had no adverse effect on outcome in 763 assisted reproduction cycles. Human Reprod. 2000;15(9):1903-1908.

19. Huang N, Situ B, Chen X, Liu J, Yan P, Kang X. Meta-analysis of estradiol for luteal phase support in in vitro fertilization/intracytoplasmic sperm injection. Fertil Steril. 2015;103:367-373. doi:10.1016/j.fertnstert.2014.10.029

Authors' Contribution:

Dr. Rehana Rehman: Designed the study, collected the samples and drafted the manuscript.

Dr. Sundus Tariq: Data entry and analysis, layout design, manuscript writing

Dr. Saba Tariq: Data analysis, table’s formulation and manuscript writing

Dr. Muhammad Faisal Hashmi: Contributed in literature review, proof reading and correction

Dr. Mukhtiar Baig: Helped in drafting, reviewing and editing the final manuscript.