DOI: 10.7860/JCDR/2018/37348.12093

Original Article

Obstetrics and Gynaecology Section

Is Luteal Phase Estradiol Supplementation Beneficial in Long Agonist IVF-ET Cycles? First Prospective Randomised Controlled Study from Indian Subcontinent

AISHWARYA KAPUR[1], SUDHA PRASAD[2], ASHOK KUMAR[3]

ABSTRACT

Introduction: The role of addition of estradiol to progesterone as luteal phase support in women undergoing In Vitro Fertilization (IVF) is not yet clear.

Aim: To determine the efficacy of oral estradiol valerate in the luteal phase of IVF cycles.

Materials and Methods: The randomized controlled study was done in the IVF centre of a Medical college. Total 150 women undergoing IVF were recruited, randomized in two groups, study (n=75) and control (n=75) using computerized randomization. Luteal phase support was started on day of Embryo Transfer (ET) : Study group-Estradiol valerate, 2mg bid orally+ Micronized progesterone, 400mg, bid vaginally and Control group: only Micronized progesterone, 400mg, bid vaginally. Blood samples for estimation of serum E2 were collected on day of oocyte retrieval, day of ET, three days after ET and on the day of confirmation of biochemical pregnancy. Statistical analysis was done using SPSS 16.0, $p<0.05$ was considered significant.

Results: Overall pregnancy rate was 38.7% (58/150): study group (41.3%), control group (36%) (p-value = 0.50). Significantly higher E2 levels were found in women who conceived as compared to those who did not conceive.

Conclusion: Although estradiol supplementation did not improve pregnancy rates significantly, but it certainly improved the hormonal profile in the study group compared with the control group.

Keywords: Estradiol valerate, Ethinyl estradiol levels, Mid luteal, Pregnancy rate, Progesterone

INTRODUCTION

Gonadotropin Releasing Hormone (GnRH) agonists, used for down-regulation in long agonist In Vitro Fertilization (IVF) cycles, work by preventing premature surges of endogenous Luteinizing Hormone (LH) through pituitary suppression, allowing time for a larger number of oocytes to reach maturity prior to harvesting. Even though the agonist treatment ends abruptly on day of Human Chorionic Gonadotropin (hCG) trigger, endogenous LH secretion can remain suppressed for as long as 10 days after treatment with a long acting GnRH agonist ends. Due to prolonged suppression of endogenous LH secretion, luteal function is frequently inadequate in amount and duration and subsequent progesterone and estradiol secretion is abnormal. Without proper progesterone or oestrogen support, endometrial receptivity is compromised, leading to decreased implantation and pregnancy rates. If luteal phase support is not provided in assisted reproduction technique cycles, the serum estradiol and progesterone levels drop, thus leading to a decrease in implantation rates and pregnancy rates.

Theoretically, hCG might offer advantages over other forms of luteal support because it stimulates the corpus luteum, both estradiol and progesterone levels are increased, along with any other factors which may facilitate implantation. However in comparative trials, its efficacy appears no greater than progesterone and several studies have demonstrated that hCG supplementation increases the risk of ovarian hyperstimulation syndrome.

The only consensus is that luteal phase supplementation improves outcomes in IVF cycles. Numerous clinical trials have compared clinical, ongoing, or delivered pregnancy rates and spontaneous miscarriage rates between groups receiving treatment with different luteal phase support regimens, with varying results. Progesterone supplementation during luteal phase is a routine treatment throughout the world with different routes and doses of administration [1].

The corpus luteum produces not only progesterone but also estradiol during the luteal phase. This has led to emergence of concept of addition of estradiol during luteal phase in IVF cycles.

The ideas about using estradiol for luteal phase support are conflicting; some reports have favoured the addition of estradiol supplementation [2-5], while others have failed to observe any beneficial effect [6-10]. Hence the role of addition of estradiol to progesterone as luteal phase support in women undergoing IVF is not yet clear. Also, no such study has been done in Indian population till now. To the best of our knowledge, our study is the first study being done in Indian population, to determine efficacy of estradiol along with progesterone as luteal phase support in women undergoing IVF. The present study was attempted to determine the effect of additional luteal phase support with estradiol on the hormonal profiles and pregnancy rates in women undergoing IVF.

MATERIALS AND METHODS

Patient Population: The study was a randomized controlled trial conducted in the IVF and Reproductive Biology Centre of a tertiary care institute and medical college of Delhi from October 2011 to March 2013. Sample size was calculated as 150, it was the sample size of convenience.

Out of 187 women screened, 150 women fulfilling the inclusion and exclusion criteria of the study were recruited and randomly divided into two groups of 75 women each (using computerized randomization).

The inclusion criteria were: 1) Age 20 to 39 years, 2), Women undergoing IVF by Long agonist protocol.

The exclusion criteria was: 1) Serum Ethinyl Estradiol (E2) level >3000 pg/mL on day of oocyte retrieval {because of risk of Ovarian Hyperstimulation Syndrome (OHSS)}; 2) Diminished ovarian reserve (Day 2 serum Follicle Stimulating Hormone(FSH)> 10 mIU/mL);

3) Endometriosis greater than stage 2; 4) Severe male factor (<5 million/mL motile spermatozoa); 5) Endocrine disorders; 6) Medical or surgical illness.

Ethical clearance was taken from the institutional ethical committee vide letter number F.No.11/IEC/MAMC/2011/246. Trial was registered with Clinical Trial Registry of India with the number CTRI/2017/10/010083. Written informed consent was obtained from all the women enrolled in the study.

Ovulation Induction: All women underwent controlled ovarian hyperstimulation by the "long" agonist protocol. Down regulation of the cycle was achieved by injection Leuprolide acetate (Sun Pharmaceuticals, India) 0.5 mg per day, subcutaneously, starting from day 21 of menstrual cycle. On the second day of next cycle, controlled ovarian stimulation was started using recombinant or urinary gonadotropins or human menopausal gonadotropins. The starting dose was 225 IU/ day of recombinant FSH (Bharat Serums and Vaccines Limited, India). The response to stimulation was monitored with serial measurements of serum estradiol and trans vaginal ultrasound imaging of ovarian follicles. Most women required a total of 9-12 days of stimulation. It was aimed to have at least two follicles measuring 17-18 mm in mean diameter, ideally accompanied by a few others in the 14-16 mm range, and a serum estradiol concentration that is consistent with the overall size and maturity of the cohort (approximately 200 pg/mL per follicle measuring 14 mm or greater). Once the targeted thresholds of response were met, hCG (10000 IU) was administered subcutaneously to induce follicular maturation. Oocyte retrieval was performed after 34-36 hours of hCG trigger, transvaginal ultrasound guided aspiration under general anesthesia being the technique. Fertilization was achieved by conventional microinsemination or by ICSI in case of male factor. Embryo transfer (ET) was performed three days after oocyte retrieval; transcervical ultrasound guided, using Sydney IVF Embryo Transfer Catheter Set (K-JETS-7019-SIVF, manufactured by Cook's Medical, USA). Urine Pregnancy Test (UPT) and serum level of beta hCG was done 14 days after ET. Positive UPT and /or serum beta hCG more than 50 mIU per mL indicated successful implantation. Women with positive UPT were followed up for detection of cardiac activity after two weeks.

Luteal Phase Support: Luteal phase support was started on the day of ET in both groups of women as follows:

Study group: Estradiol valerate, 2mg tablet orally, twice daily (BayerZydus Pharmaceuticals Private Limited, India) and Micronized progesterone, 400mg, softgel capsule, vaginally, twice daily (Sun Pharmaceuticals, India).

Control group: Micronized progesterone, 400mg, softgel capsule, vaginally, twice daily (Sun Pharmaceuticals, India).

Specimen Collection and Hormone Analysis: Blood samples (4 mL) for estimation of serum E2 was collected in plain vials on the day of oocyte retrieval (here after referred to as Day 0), on the day of ET (Day 3), three days after ET (Day 6), on the day of confirmation of biochemical pregnancy (Day 17). Serum levels of E2 were estimated by Radio Immuno Assay.

STATISTICAL ANALYSIS

Statistical analysis of difference between study and control group was done using Statistical Package for Social Sciences (SPSS) Version 16.0. The two groups were analyzed using the Pearson chi-square test, Mann-Whitney U test and student t-test, as indicated. The p-value of less than 0.05 was considered significant.

RESULTS

There was no difference in the two groups with respect to demographic features, duration, type and etiological factors for infertility [Table/Fig-1].

The overall pregnancy rate in the study was 38.7% (58/150). The pregnancy rate was observationally higher in the study group (41.3%) as compared to the control group (36%) but the difference was not statistically significant (p-value = 0.50).

Variable	Study Group (E+P) N=75	Control Group (Only P) N=75	p-value
Age (years)	31.62±4.15	30.68±4.01	0.16
Duration of infertility (years)	7.98±4.40	8.38±4.10	0.58
Type of Infertility (%) (Primary/Secondary)	70.7/29.3	78.7/21.3	0.26
Aetiological factors for infertility (%)			
Tubal factor	17.3	12	0.17
Male factor	45.3	62.7	
Unexplained	30.7	22.7	
Endometriosis	6.7	2.7	

[Table/Fig-1]: Characteristics of the two study groups.

The serum level of E2 in the study and control group on days 0, 3, 6, 17 were compared, as depicted in [Table/Fig-2] and also in women who conceived and those who didn't conceive, in [Table/Fig-3].

	Study Group (E+P) N=75	Control Group (Only P) N=75	p-value
E2 level (pg/mL)			
Day 0	2028.16±480.26	2059.95±511.23	0.47
Day 3	1697.78±434.91	1633.78±434.82	0.40
Day 6	1425.08±389.02	1204.44±312.50	<0.001
Day 17	460.93±418.19	312.98±268.43	0.03
Percentage E2 decline from Day 0 to Day 6 (%)	29.98±8.61	40.92±8.82	<0.001
Day 0 E2: Day 6 E2 ratio	1.45±0.22	1.73±0.28	<0.001

[Table/Fig-2]: Comparison of Serum E2 levels in the two groups.

	Pregnant (N=58)	Non Pregnant (N=92)	p-value
E2 level (pg/mL)			
Day 0	2120.57±538.48	1995.82±461.31	0.23
Day 3	1760.41±465.46	1606.17±405.27	0.06
Day 6	1403.23±376.67	1258.98±354.31	0.02
Day 17	541.80±498.01	289.34±171.94	<0.001
Percentage E2 decline from Day 0 to Day 6 (%)	33.29±9.31	36.81±10.66	0.02
Day 0 E2: Day 6 E2 ratio	1.53±0.25	1.63±0.30	0.03

[Table/Fig-3]: Comparison of Serum E2 levels in pregnant and non pregnant women.

The percentage fall in E2 level from Day 0 to Day 6 was significantly lower in study group, as compared to control group. Also, the women who conceived had a significantly lower percentage fall in E2 levels, as compared to the women who did not conceive. The ratio of Day 0 E2 level to Day 6 E2 level was significantly lower in the study group compared to the control group. Also, the women who conceived had a significantly lower Day 0 E2: Day 6 E2 ratio compared to those who did not conceive [Table/Fig-2].

DISCUSSION

Oestrogen during luteal phase has a modulatory effect on the endometrial progesterone receptor concentration and serves to replenish and maintain a sufficient level of progesterone receptors to mediate and complete progesterone response. This has been proved by studies on the effect of luteal phase oestrogen antagonism on endometrial development and luteal function in women. As

mentioned earlier, the results of addition of estradiol as luteal phase support are conflicting. Even the results of meta-analyses are also not clear. After thorough search of literature, we did not find any study done in Indian population in this regard. Kolibianakis WM et al., Gelbaya TA et al., and Jee BC et al., suggested that the addition of E2 to P for luteal phase support does not improve IVF outcomes [11-13]. On the other hand, meta-analysis done by Huang N et al., and Pinhero LMA et al., reported conflicting results and suggested further trials to determine the beneficial effect of estradiol administration in luteal phase [14,15]. Another meta-analysis by Zhang XM et al., suggested improved pregnancy rates with addition of oestrogen [16]. In our study, the pregnancy rate was observationally higher in the study group (41.3%) as compared to the control group (36%) but the difference was not statistically significant.

The limiting factor in the success of IVF is implantation, which involves two major participants, embryos and endometrium. Endometrial development is driven by oestrogen secretion from the ovaries. Serum levels of estradiol and progesterone decline in the mid luteal phase in IVF cycles in which pituitary suppression is used during controlled ovarian hyper stimulation. This decline has been found to adversely affect implantation [17].

Significantly higher E2 levels were found on Day 6 and 17 in study group and in women who conceived. Ghanem ME et al., and Var T et al., also reported significantly higher E2 levels in E+P group compared to P only group on day 7 after hCG trigger [2,4].

The levels of serum E2 decrease in the luteal phase. It has been shown that this decline adversely affects implantation. It is found that lower luteal phase E2 level 10 days after oocyte retrieval lowers the chances of conception and the magnitude of decline in estradiol concentrations after oocyte retrieval may be important in predicting IVF success. It is postulated that endometrial integrity may become compromised when a dramatic drop in estradiol occurs by the mid-luteal period. The percentage decline in estradiol levels from the day of oocyte retrieval to the day of implantation was significantly lower in study group, as compared to control group and also in women who conceived as compared to those who did not conceive.

Ghanem ME et al., in their study have tried to predict the pregnancy rate according to the E2 peak to midluteal E2 ratio [2]. In our study, the Day 0 E2: Day 6 E2 ratio was significantly lower in the study group, compared to control group. Also, the women who conceived had a significantly lower Day 0 E2: Day 6 E2 ratio compared to those who did not conceive.

LIMITATION

It was found that although estradiol supplementation in the luteal phase did not improve pregnancy rates significantly, but it certainly improved the hormonal profile in the study group compared with the control group, hence a study with larger number of women is required to show a significant difference in pregnancy rate.

CONCLUSION

It was concluded that estradiol supplementation in luteal phase of IVF cycles in beneficial. It is also postulated that a further increase in dose of estradiol valerate to 6 mg/day may increase the pregnancy rate significantly, although a properly designed randomized controlled study with dose stratification (2 mg/day, 4 mg/day and 6 mg/day) needs to be conducted to prove this hypothesis. Also, it is postulated that beginning of estradiol supplementation from the day of oocyte retrieval may result in even better hormonal profiles and eventually translate into higher pregnancy rates, but this needs to be proven by a randomized controlled study with a larger sample size.

ACKNOWLEDGEMENTS

All the patients who willingly participated in the study. All the seniors, colleagues and staff members for their guidance, help and cooperation.

REFERENCES

[1] Lukaszuk K, Liss J, Lukaszuk M , Maj B. Optization of estradiol supplementation during the luteal phase improves the pregnancy rate in women undergoing in vitro fertilization - embryo transfer cycles. Fertil Steril. 2005;83:1372-76.
[2] Ghanem ME, Sadek EE, Elboghdady LA, Helal AS, Gamal A, Eldiasty A, et al .The effect of luteal phase support protocol on cycle outcome and luteal phase hormone profile in long agonist protocol intracytoplasmic sperm injection cycles: a randomized clinical trial. Fertil Steril. 2009;92:486-93.
[3] Elgindy EA, El-Haieg DO, Mostafa MI, Shafiek M. Does luteal estradiol supplementation have a role in long agonist cycles? Fertil Steril. 2010;93:2182-88.
[4] Var T, Tonguc EA, Doğanay M, Gulerman C, Gungor T, Mollamahmutoglu L. A comparison of the effects of the three different luteal phase support protocols on in vitro fertilization outcomes: a randomized clinical trial. Fertil Steril. 2011;95(3):985-89.
[5] Kutlusoy F, Guler I, Erdem M, Erdem A, Bozkurt N, Biberoglu EH, et al. Luteal phase support with estrogen in addition to progesterone increases pregnancy rates in in vitro fertilization cycles with poor response to gonadotropins. Gynecol Endocrinol. 2014;30(5):363-66.
[6] Serna J, Cholquevilque JL, Cela V, Martinez-Salazar J, Requena A, Garcia-Velasco JA. Estradiol supplementation during the luteal phase of IVF-ICSI women: a randomized, controlled trial. Fertil Steril. 2008;90:2190-95.
[7] 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;89:554-61.
[8] Moini A, ZadehModarress S, Amirchaghmaghi E, Mirghavam N, Khafri S, Reza Akhoond M, et al. The effect of adding oral oestradiol to progesterone as luteal phase support in ART cycles - a randomized controlled study. Arch Med Sci. 2011;7:112-16.
[9] Lin H, Li Y, Li L, Wang W, Zhang Q, Chen X, et al. Oraloestradiol supplementation as luteal support in IVF/ICSI cycles: a prospective, randomized controlled study. Eur J Obstet Gynecol Reprod Biol. 2013;167:171-75.
[10] Ismail Madkour WA, Noah B, Abdel Hamid AM, Zaheer H, Al-Bahr A, Shaeer M, et al. Luteal phase support with estradiol and progesterone versus progesterone alone in GnRH antagonist ICSI cycles: a randomized controlled study. Hum Fertil (Camb). 2016;19(2):142-49.
[11] Kolibianakis EM, Venetis CA, Papanikolaou EG, Diedrich K, Tarlatzis BC, Griesinger G. Estrogen addition to progesterone for luteal phase support in cycles stimulated with GnRH analogues and gonadotrophins for IVF: a systematic review and meta-analysis. Hum Reprod. 2008;23:1346-54.
[12] Gelbaya TA, Kyrgiou M, Tsoumpou I, Nardo LG. The use of estradiol for luteal phase support in in vitro fertilization/intracytoplasmic sperm injection cycles: a systematic review and meta-analysis. Fertil Steril. 2008;906:2116-25.
[13] Jee BC, Suh CS, Kim SH, Kim YB, Moon SY. Effects of estradiol supplementation during the luteal phase of in vitro fertilization cycles: a meta-analysis. Fertil Steril. 2010;93:428-36.
[14] Huang N, Situ B, Chen X, Liu J, Yan P, Kang X, et al. Meta-analysis of estradiol for luteal phase support in In vitro fertilization/intracytoplasmic sperm injection. Fertil Steril. 2015;103(2):367-73.
[15] Pinheiro LMA, Cândido PDS, Moreto TC, Almeida WGD, Castro EC. Estradiol use in the luteal phase and its effects on pregnancy rates in IVF cycles with GnRH antagonist: a systematic review. JBRA Assist Reprod. 2017;21(3):247-50.
[16] Zhang XM, Lv F, Wang P, Huang XM, Liu KF, Pan Y, et al. Estrogen supplementation to progesterone as luteal phase support in patients undergoing in vitro fertilization: systematic review and meta-analysis. Medicine (Baltimore). 2015;94(8):e459.
[17] Bai X, Zhang Y, Liu S, Qu D, Su H, Ren H, et al. The decline in serum estradiol on the second day after oocyte retrieval affects the outcome of IVF/ICSI-ET treatment in high ovarian responders. Gynecol Endocrinol. 2017;33(6):452-57.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Obstetrics and Gynaecology, Lady Hardinge Medical College, New Delhi, India.
2. Director Professor, Department of Obstetrics and Gynaecology, Maulana Azad Medical College, New Delhi, India.
3. Director Professor, Department of Obstetrics and Gynaecology, Maulana Azad Medical College, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Aishwarya Kapur,
Assistant Professor, Department of Obstetrics and Gynaecology, Lady Hardinge Medical College, New Delhi-110001, India.
E-mail: aish9kapur@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jun 03, 2018**
Date of Peer Review: **Jul 11, 2018**
Date of Acceptance: **Jul 21, 2018**
Date of Publishing: **Oct 01, 2018**