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July 2014

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Impact of peak/mid luteal estradiol on pregnancy outcome after intracytoplasmic sperm injection

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Abstract

Objective: To compare peak to mid estradiol ratio with the probability of successful conception after intra-cytoplasmic sperm injection.

Method: The quasi-experimental study was conducted in an infertility clinic at Islamabad from June 2010 till August 2011, and comprised couples subjected to intra-cytoplasmic sperm injection. Down-regulation of ovaries was followed by calculated stimulation, ovulation induction, oocytes retrieval, intra cytoplasmic sperm injection, in vitro maturation of embryos and finally blastocysts transfer. Serum estradiol was measured by enzyme-linked immunosorbent assay on ovulation induction day and the day of embryo transfer. Failure of procedure was detected by beta human chorionic gonadotropin5-25mIU/ml (Group I; non-pregnant).Females with beta human chorionic gonadotropin>25mIU/ml and no cardiac activity after 4 weeks of transfer were placed in Group II (pre-clinical abortion), and confirmation of foetal heart in the latter comprised Group III (clinical pregnancy). Data was analysed using SPSS 15.

Results: Of the 323 couples initially enrolled, embryo transfer was carried out in 282(87.3%) females. Clinical pregnancy was achieved in 101(36%) of the cases, while 61(21.63%) had pre-clinical abortion, and 120(42%) remained non-pregnant. The peak/mid-luteal estradiolratio was low (2.3) in patients who had high oocyte maturity (p=0.001) and fertilisation rate (p=0.003) compared to non-pregnant patients with high peak/mid-luteal estradiolratio (2.56).

Conclusion: High peak estradiol with maintenance of optimal levels in mid-luteal phase is required for implantation of fertilised ovum and accomplishment of clinical pregnancy.

Keywords: Assisted reproductive treatment, Controlled ovarian stimulation, Embryo transfer, Gonadotrophin-releasing hormone agonists, Intra-cytoplasmic sperm injection, Peak estradiol, mid-luteal estradiol, ratio of peak/mid luteal E2. (JPMA 64: 780; 2014)

Introduction

Assisted reproduction is the scientific assistance provided to infertile couples. The procedures represent a coalescence of advancement in physiology, endocrinology, pharmacology, diagnostic technology and clinical care. The most popular procedures employed in assisted reproductive clinics (ARC) comprise in vitro fertilisation and intra-cytoplasmic sperm injection (ICSI).[1]

ICSI comprises specialised treatment plan in which a couple is involved for duration of at least 6 weeks. The extensive, expensive and rigorous treatment of at least six weeks of couple's involvement unfortunately ends up

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with a maximum success rate of 25-30%. The negative pregnancy test comes as a setback for the couple as well as the infertility specialists. There is a long list of maternal and foetal factors that could be blamed for the failure of implantation during the narrow window of implantation. Detection of one of these factors may help in selection of couples, improvement of treatment plans and prediction of ICSI outcomes.[2]

ARCs do their best in making all possible attempts to improve success rates.[3] The peak estradiol (E2) measured on the day of human chorionic gonadotrophin (hCG) administration is associated with development and maturation of follicles and better outcome after in vitro fertilization (IVF)-ICSI procedures.[4,5] The exact role of mid-luteal E2 is subject to differences on the basis of estimation on different days of the luteal phase. Some studies have emphasised the role of the ratio of peak-mid-luteal E2 on pregnancy outcome,[6] few found that ratios greater than five were associated with an adverse outcome.[7] The objective of this study was to find an association of E2 ratio in follicular and mid-luteal phase

with pregnancy outcome.

Subjects and Methods

The quasi-experimental study was conducted in an Islamabad-based infertility clinic from June 2010 to August 2011. The sample size was calculated using the formula[8]

$$n= Z2 \ pq/e2 = (1.96)2 \ (0.3) \ (0.7) / (0.05)2 = 323$$

where 'e' is the margin of error.

According to literature, successful pregnancy rate of ICSI is around 25-30%. By taking this probability, we had drawn a sample size of 323 patients at 95% confidence interval (CI) with 5% margin of error. Using convenience sampling,323 consenting couples were enrolled meeting the inclusion criteria of female age 18-41, duration of infertility more than 2 years, both ovaries present with no morphological abnormalities, normal menstrual cycle (25-35 days), body mass index (BMI)of 18-27 kg/m², basal Follicle-stimulating hormone(FSH) (day 2) serum level <10IU/mL, elected for long protocol with Gonadotrophin releasing hormone agonist (GnRha), stimulated with injection of recombinant follicle stimulating hormone(rFSH; Puregon) and kept on progesterone support with 400mg cyclogest pessaries. Females on GnRh antagonist, short-down regulation with GnRH agonist and ICSI with sperm retrieval by testicular biopsy were excluded.

The subjects were down-regulated with daily injection DecaPeptyl (GnRha) from mid-luteal phase of preceding cycle followed by controlled ovarian stimulation (COS) with rFSH (injection Puregon S/C) from 2nd to third day of cycle for 13±2 days. Maturity of follicle was assessed by Trans-vaginal scan (TVS) starting from 5th day of COS till decision of Oocyte pick-up (OPU). Ovulation induction (OI) with intra-muscular injection of hCG (Pregnyl 10,000 IU) was performed with preponderance of mature follicles; size ≥20mm. The venous samples were taken for estimation of peak E2 on this day. Oocytes were retrieved 36 hours after OI by vaginal ultrasound probe with 16G adapter and double lumen oocyte aspiration needle on 14th, 15th or 16th day of the COS. Collected oocytes were stripped and transferred to the incubator for about 1-2 hours prior to ICSI procedures. Semen analysis was performed by strict Kruger's criteria.[9] ICSI by micro-injections of spermatozoa was performed at right angles to the position of polar body under the microscope. Fertilised embryos (presence of two pro-nuclei; 2PN) was assessed and embryos graded on alternate days for their developmental characteristics in vitro; cleavage till differentiation into distinct cell types with formation of fluid-filled cavity (blastocysts). Embryo transfer (ET) of

blastocysts was done seven days after OI by Sims-Wallace Embryo Replacement Catheter under ultrasound guidance. Luteal support was maintained by progesterone vaginal pessaries (Cyclogest 400mg) twice a day from the day of OPU. Samples for mid-luteal estimation of E2 were taken on the day of ET, on day 19 or 20 of the cycle.

Single serum beta hCG measurement was performed on specimens obtained by peripheral venipuncture 14 days after egg collection as the outcome marker. TVS was performed at 5-week gestation to identify clinical pregnancy from pre-clinical abortion. On the basis of beta hCG and TVS, results were categorised into groups; I, non-pregnant with beta hCG 5-25ml U/ml; II, pre-clinical abortion beta hCG>25 m IU/ml with no foetal cardiac activity on TVS; and III, clinical pregnancy with beta hCG>25 m IU/ml and cardiac activity confirmed by TVS.[10] The Peak/mid-luteal E2 in was calculated in all three groups.

Pregnancy outcomes and the associated rates were defined using standard definitions as follows: Oocyte recovery rate was the number of oocytes retrieved in comparison to number of follicles calculated.[11] The percentage of mature oocytes was oocyte maturity rate. Fertilization rate (FR) was proportion of micro-injected oocytes resulting in 2PN formation.[12] The implantation rate (IR) was the number of gestational sacs visualised on TVS divided by the number of embryos transferred.[13] Pregnancy rate (PR) was calculated by the presence of an intrauterine gestational sac observed on TVS per number of patients in the cycle.[11]

Data was entered into MS Excel and exported to SPSS 15 for statistical analysis. Qualitative variables (age group) were summarised in terms of frequencies and percentages, and mean standard deviation (SD) was used for continuous/quantitative variables. For inferential analysis, continuous variables were checked whether they followed normal distribution. This inference was made using Kolmogorov-Smirnov's test and if P value came out to be more than 0.05, the variables were considered to be normally distributed. Results of normal variables in outcome groups were compared by analysis of variance (ANOVA), whereas Kruskal Wallis test was applied for non-normal variables. In all statistical analysis, p<0.05 was considered statistically significant.

Results

Of the 323 couples initially enrolled, embryo transfer (ET) was carried out in 282(87.3%) females. Clinical pregnancy was achieved in 101(36%) of the cases, while 61(21.63%) had pre-clinical abortion, and 120(42%) remained non-pregnant. The overall mean age of the females was

Table-1: Demographic and cycle characteristics.

Variables	Group I: Non- pregnant (120) Peak /mid luteal E2 ratio 2.56	Group II: Preclinical abortion (61) Peak /mid luteal E2 ratio 2.34	Group III: Clinical pregnancies (101) Peak /mid luteal E2 ratio 2.3	P value
Age (Years)	31.95±4.61	32.31±4.70	32.17±4.71	0.871
Duration of infertility* (Years)	6.89±3.78	6.96±3.82	7.46±4.00	0.529
Body Mass Index (kg/m2)	24.81±3.68	24.24±3.64	23.56±3.63	0.042
Antral follicular count	16.53±1.15	13.61±3.27	13.07±2.55	0.001
Ovarian Volume by Ultrasound (cm3)*	12.07±3.33	11.58±2.82	10.90±2.78	0.004
Pre Ovulatory Follicle	7.17±1.40	8.23±2.47	8.31±1.70	0.000
No of oocytes/patient	7.09±1.32	8.02±2.11	8.21±1.52	0.000
No of oocytes Metaphase II	6.28±1.94	7.3±2.05	8.03±1.44	0.000
No of oocytes fertilized	5.29±1.59	6.07±1.64	6.65±1.07	0.000
Number of puregons in one day	4.2±0.76	3.90±0.64	3.83±0.486	0.000
Total number of puregons	60.30±10.96	56.43±7.88	53.99±5.72	0.000
Endometrial thickness	5.967±2.691	9.033±2.345	11.455±2.037	0.000
Peak Estradiol on the day of hCGpg/ml*	2181.00±301.56	2261.00±240.23	2529.05±193.99	0.000
Estradiol in mid luteal phase pg/ml*	858.66±97.21	979.79±127.36	1109.46±136.28	0.000

hCG: Human chorionic gonadotropin
Values expressed as Mean ± standard deviation
Normal variables of clinical pregnancy group compared with non-pregnant by one way of analysis of variance
*Non normal variables of clinical pregnancy compared with non pregnantgroup by Kruskal Wallis test.

Table-2: Comparison of reproductive rates.

Rates in percentages	Group I: Non- pregnant (120) Peak /mid luteal E2 ratio 2.56	Group II: Preclinical abortion (61) Peak /mid luteal E2 ratio 2.34	Group III: Clinical pregnancy (101) Peak /mid luteal E2 ratio 2.3	P value
Oocyte retrieval rate	99.23±4.398	98.28±3.337	99.15±2.577	0.217
Oocyte maturity rate	89.15±23.023	91.82±16.55	98.14±6.037	0.001
Fertilisation rate	75.07±18.67	76.73±14.355	81.71±6.814	0.003

Values expressed as Mean ± standard deviation
Clinical pregnancy group compared with non-pregnant group by Kruskal Wallis test
Oocyte recovery rate (%)= Total number of oocytes retrieved/Total number of pre ovulatory follicles × 100
Oocyte maturity rate (%)= Total number of metaphase II oocytes / Total number of oocytes retrieved × 100
Fertilisation rate (%)= Total number of 2 pronuclei /Total number of oocytes microinjected × 100.

31.55±4.62 years with duration of infertility being 7.48±3.68 years. Female cause of infertility was found in 70(25%) patients, 36 (13%) had unexplained infertility, male infertility was 113(40%) and both were responsible in 63(22%) cases. Females had a mean BMI of 23.55±3.86kg/m² with endometrial thickness of 8.58±3.41. Total number of puregons used/patient were 60±3.07, while used/day were 4.00±0.21. Oocytes retrieved per patient were 19.35±0.52 (oocyte recovery rate was 97.21±6.8) out of which 15.07±0.49 were fertilised (FR: 61.01±1.71), 10.57±0.42 cleaved (cleavage rate: 53.05±22.95) and 1.63±0.03 blastocysts transferred (31.20±2.99).

Comparison of peak/mid-luteal ratio in all groups was calculated (Figure). In the non-pregnant group it was 2.5, it was 2.34 in the group of pre-clinical abortions, and it was 2.30 in the group that had clinical pregnancies (Table-1). The last group with low peak/mid-luteal ratio
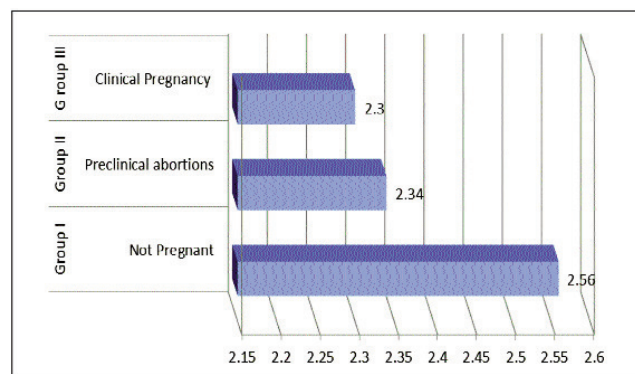
Figure: Comparison of peak/mid-luteal Estradiol ratiosin study Groups.

demonstrated better oocyte quality parameters and endometrial thickness with less number of rFSH injections. Peak E2 levels were the highest in the same group III compared to the other two (Table-2). IR of 83.66±22.84 in Group III was also better than Group II 4.92±21.804.

Discussion

The task of E2 released in the follicular phase is responsible for follicular development as well as endometrial proliferation and hyperplasia of both glandular and stromal components.[14] This is made possible by assembly of specific proteins, growth factors, hormones in addition to up-regulation of hormone receptors. The estimation of changes in E2 secreted during proliferative and secretory phase of the endometrial cycle with its impact on follicular growth, endometrial development and uterine receptivity may thus help in consideration of E2 ratios for better outcome and improvement in clinical pregnancy rates after ICSI.

The role of E2 levels on the day of hCG administration for a positive pregnancy outcome is not accepted by a few studies.[15-17] In our study, Group III patients with peak/mid-luteal E2 ratio of 2.3, patients had highest peak E2 in which greater number of oocytes were retrieved with low dose of rFSH (puregon)which is comparable to other studies.[6,18] Similar results were reported by Kara et al.[19] Improvement with oocyte and embryo quality parameters with high peak E2 was observed by a few others.[18] Higher peak E2 levels with better pregnancy rates were observed by others.[4,5,13] On the contrary, few studies suggested a damaging role of high E2 levels on the day of hCG administration for pregnancy achievement.[20,21] In our patients, high peak E2 helped in better oocyte maturity, fertilisation and cleavage of embryos.

In the stimulation cycles of ICSI with GnRha therapy, 2-3 weeks are required for the re-establishment of pituitary function.[13] In this period, corpus luteum rescues production of E2 and progesterone (P) in optimal amounts to maintain endometrial receptivity during decidualisation.[22] Quite a few groups have observed that mid-luteal E2 levels do not affect IVF outcome.[5,21] We noticed an increase in E2 in the luteal phase of pregnant women which is in agreement with the reports of several research groups.[22-24] Fujimoto et al. observed a lower pregnancy rate in women with decreased E2 levels in the late mid-luteal phase in IVF cycles.[23] Decrease in luteal E2 of Group I patients was associated with decreased intercessory prayer (IP) and PR in our study, which is analogous to studies done by others.[13,25]

Implantation failed to occur in the luteal phase of Group I patients due to decline in function of corpus luteum with fall in mid-luteal E2 and release of Inhibin.[22] The decline in mid-luteal E2 and P levels were found to be associated with failure of conception.[11] The decrease in E2 levels during mid-luteal phase was more in non-pregnant females compared to pregnant females (p<0.001) with ratio of 2.3 associated with clinical pregnancy and >2.3 was seen in non-conception cycle. Conflicting results of peak and mid-luteal E2 levels have been reported between conception and non-conception cycles which could not reach statistical significance.[21] It was later proposed that lack of statistical significant difference by Ng et al. was low pregnancy rates in those groups.[26] The ratio of peak/mid-luteal E2 level higher than 2.5 predicted impaired implantation rate and pregnancy outcome.[7]

Implantation of embryo depends on quality of the embryos and endometrial receptivity.[3] Since blastocysts with maximum implantation potential were used in our study, hence better implantation was attributed to endometrial receptivity provided by the preservation of hormones in the luteal phase. The increased E2 produced by growing follicles increases endometrial receptivity which renders it susceptible to apposition, attachment and apposition for the encroaching blastocyst.[27] This receptivity is required to be maintained in the luteal phase for a successful outcome.[28] The results of our study highlighted that increased endometrial thickness was seen in women who had a peak/mid-luteal E2 ratio of 2.3.

The P levels are not mentioned in this study owing to the biases with the use of progesterone pessaries after OPU. Various studies done on estimation of peak to mid luteal E2 ratios diverge on the basis of time for the estimation of luteal E2. In our study, serum E2 level was estimated on the day of blastocyst transfer, which is 7 days after OI. This estimation was done 11 days after OI by Ganesh et al. who found that luteal E2 levels were a promising marker for successful pregnancy.[22] This being the first study in Pakistan determined a cut-off value for the E2 ratios, which will enable Assisted Reproductive Technology (ART) to visualise a conception by estimation of peak/mid-luteal E2 ratio.

Conclusion

The ratio greater than 1 in all outcome groups for investigating the impact of peak/mid-luteal estradiol on pregnancy outcome after ICSI indicated that the value of peak E2 was always found higher than mid-luteal E2. The peak and mid-luteal E2 was maximum in patients who acquired clinical pregnancy compared to pre-clinical abortions and non-pregnant group. Furthermore, a ratio

of 2.3 in clinical pregnancy group highlighted the importance of mid-luteal E2 in the maintenance of pregnancy.

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