

Prediction of success in intracytoplasmic sperm injection (ICSI) by estimation of serum Estradiol/Progesterone ratio on the day of embryo transfer

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Abstract

Objective: To compare estradiol-to-progesterone ratio with pregnancy outcome in patients of intracytoplasmic sperm injection.

Methods: The quasi-experimental study was conducted on 106 couples at an assisted reproductive clinic in Islamabad from June 2010 to August 2011. Down-regulation of females aged 18-41 with Gonadotrophin releasing hormone agonist was followed by calculated stimulation with Gonadotrophin injections (Puregon). Oocytes pickup was done 36 hours after ovulation induction by human chronic gonadotropin (hCG) (on Day 0), eggs fertilised in vitro were graded and only blastocysts were transferred on Day 5 (Ovulation induction+7 days). Serum estradiol and progesterone was measured by Enzyme-Linked Immunosorbent Assay on D0 and D5 and its values were compared in 3 groups of females; no conception beta hCG<5 mIU/ml; pre-clinical abortion with beta hCG>5 mIU/ml and no cardiac activity on trans-vaginal scan; and clinical pregnancy with beta hCG>5mIU/ml and cardiac activity on trans-vaginal scan. SPSS 15 was used for data analysis.

Results: Out of the 106 females, 12 (11.32%) had to be excluded for various reasons at different stages. The final sample stood at 94 (88.67%). Of them, 33 (35.1%) achieved clinical pregnancy, 21 (22.3%) had non-viable pregnancy, and 40 (42.6%) failed to conceive. Females who had significantly high number of oocytes and thick endometrial lining at the day of hCG administration were found to have significant rise in estradiol-to-progesterone ratio on the day of embryo transfer which correlated with a positive pregnancy test and cardiac activity on trans-vaginal scan ($p < 0.05$).

Conclusion: The success of intracytoplasmic sperm injection could be predicted by good number of oocytes that secrete large amount of estradiol, increased thickness of endometrial lining, and a high estradiol-to-progesterone ratio on the day of embryo transfer.

Keywords: Intracytoplasmic sperm injection, Assisted reproductive treatment, Controlled ovarian stimulation, Gonadotrophin-releasing hormone agonists, Embryo transfer, Estradiol, Progesterone. (JPMA 63: 609; 2013)

Introduction

In vitro fertilization (IVF) or Intracytoplasmic sperm injection (ICSI) is a highly developed practice of Assisted Reproductive Treatment (ART) carried out in a period of four to six weeks from the initiation of ovulation (OT) to embryo transfer (ET).¹ In Assisted reproductive Clinics (ARCs), failure of implantation is attributable to a long list of factors ranging from maternal age, poor ovarian reserves, sperm quality, pitfalls in treatment procedures for follicular maturity, fertilisation of zygote, embryonic development in vitro and endometrial preparation for apposition, to adhesion and implantation of invading blastocysts.^{1,2}

Controlled ovarian stimulation (COS) carried out in ARC aims to sustain optimal levels of progesterone (P) and estradiol (E2) during the implantation period, which are

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required for successful conception, by preparation of endometrial bed for the encroaching embryo.³ This is achieved by regulation of locally-produced cytokines, growth factors, homeobox transcription factors and cyclooxygenase-derived prostaglandins through autocrine and paracrine pathways.⁴ Regulation of foeto-maternal crosstalk is made possible by up-regulation of adhesion molecules either on the endometrial pinopods or on the blastocyst which approach their equivalent ligands on other epithelial surfaces.⁵ E2 in this regard synchronises endometrium lining for the reception of blastocyst implantation by a series of events which include initiation of hypertrophy and hyperplasia of the endometrium with development of P receptors in the luteal phase of the ovarian cycle.⁶ P secreted by corpus Luteum is vital for blastocyst implantation, successful conception and the continuation of pregnancy³ once it is primed by E2.⁷

The current study was planned to determine the E2-P ratio in luteal phase of ICSI female's cycle, and to relate it with no pregnancy, pre-clinical abortion, and

clinical pregnancy.

Patients and Methods

The quasi-experimental study was conducted after approval from Ethical Review Board of the Islamabad Clinic Serving Infertile Couples, from June 2010 to August 2011. Before starting this research, prior information that pregnancy rate is around 30% was used to calculate the sample size by using the following formula.⁸

$$N = Z^2 \alpha / 2 pq / e^2$$

(Where e is margin of error)

The required sample size was 96 females at 95% confidence level with 10% margin error. Using convenience sampling, 106 consenting couples were registered on the basis of inclusion criteria; female age 18-41, duration of infertility more than 2 years, both ovaries present with no morphological abnormalities, normal ovulatory cycle (25-35 days), body mass index (BMI) of 18-27 kg/m², basal follicle stimulating hormone (FSH) (day 2) serum level <10mIU/mL, selected for long protocol with Gonadotrophin releasing hormone agonist (GnRH), stimulated with injection of recombinant FSH (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 Deca Peptyl (GnRH) from Day 21 of previous cycle followed by COS by gonadotrophins (Inj Puregon I/M or S/C) from D2 to D3 of the cycle for 14 days. Maturity of follicle (20mm) was assessed by series of trans-vaginal scan (TVS) started from D5 of COS till the decision of egg collection, followed by ovulation induction (OI) with intramuscular injection of hCG (Pregnyl 10,000IU). Eggs were retrieved 36 hours after OI by vaginal ultrasound probe with 16G adapter and double lumen oocyte aspiration needle on D14, D15 or D16 of COS. Oocytes pick-up (OPU) was registered as D0 on which venous sample was taken for initial E2 and P estimation. All eggs collected were treated and then transferred to the incubator for about 1-2 hours prior to insemination by ICSI procedures. Semen analysis was performed by strict Kruger's criteria⁹ and film was prepared by Silselect gradient. 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 pronuclei; 2PN) were assessed and graded daily for their developmental characteristics in vitro; cleavage till differentiation into distinct cell types with formation of fluid-filled cavity (blastocysts). ET of blastocysts was done

five to seven days after OI by Sims-Wallace Embryo Replacement Catheter under ultrasound guidance. E2 and P were repeated by Enzyme Linked Immunosorbent Assay (ELISA) technique on ET day. Luteal support was maintained by progesterone vaginal pessaries (Cyclogest 400mg) twice a day from the day of OPU.¹⁰

Single serum beta human chronic gonadotropin (hCG) measurement was performed on specimens obtained by peripheral venipuncture 14 days after egg collection as the outcome marker.¹¹ TVS was performed at 5 weeks gestation (22 to 32 days after fertilisation)¹² to identify clinical pregnancy from pre-clinical abortion. On the basis of beta hCG and TVS, results were categorised into non-pregnant with beta hCG <5mIU/ml, preclinical abortion beta hCG >5 mIU/ml with no foetal cardiac activity on TVS, clinical pregnancy with beta hCG >5 mIU/ml and cardiac activity confirmed by TVS.

Data analysis was carried out by SPSS version 15.0. Clinical characteristics were summarised in terms of frequencies and percentages for qualitative variables (age group), mean standard deviation for continuous/quantitative variables (p level at D1, D5; E2 level at D1, D5; and beta hCG, etc). Statistical comparison was performed by using One-way analysis of variance (ANOVA) and Pearson Correlation Coefficient of beta hCG with other variables. In all statistical analysis p <0.05 was considered significant. The receiver operating characteristic (ROC) curves were developed to depict probability of true-positive results (sensitivity) as a function of false-positive results (1-specificity). Sensitivity and specificity was calculated for all determined ratios of the decision axis and combined with the area under the curve (AUC). The AUC (sensitivity/1-specificity) format approach was used to confirm test adequacy (AUC near 1) or inadequacy (AUC near 0.5).

Results

Of the 106 females initially registered, 12 (11.32%) had to be excluded at various stages. The final sample stood at 94 (88.67%).

The total included 14 (15%) in 15-25 age group; 30 (32%) in 25.5-30 years; 29 (31%) in 30.5-35 years; and 21 (22%) in the 35.5-41 years age group. When stratified by diagnostic category, 25 (26.6%) patients had tubal disease; 12 (12.76%) had endometriosis; 13 (13.8%) had polycystic ovary syndrome, 20 (21.27%) had unexplained infertility, and 24 (25.53%) had male factor infertility. For some patients, more than one infertility factor was assigned. The duration of infertility 2-5 years, 5-8 years and more than 8 years was found in 46 (49%), 36 (38%) and 12 (13%) respectively.

Out of these, 33 (35.1%) achieved clinical pregnancy; 21

Table-1: Comparison of variables in the three groups.

Variables	Patients			P-Value (by using Anova)
	Not pregnant n=33	Preclinical abortion n=21	Clinical pregnancies n=40	
Female age (years)	31.225 ± 0.782	31.571 ± 0.88	31.242 ± 0.851	0.7892
Duration of infertility (years)	5.5 ± 0.544	4.738 ± 0.509	6.515 ± 0.865	0.243337
rFSH (Puregon ampoules)	31.4 ± 8.0	30.20 ± 10.8	26.6 ± 6.8	0.224
Day of egg collection	15.075 ± 0.126	15.286 ± 0.184	14.818 ± 0.147	0.121833
No of oocytes	14.8 ± 1.067	15.857 ± 1.574	20.394 ± 1.509	0.007009*
No of oocytes fertilized PN	12.025 ± 1.032	12.286 ± 1.639	16.152 ± 1.376	0.040481*
Endometrial lining	8.955 ± 0.279	10.095 ± 0.457	10.47 ± 0.308	0.00209*
Number of blastocysts transferred	1.65 ± 0.092	1.476 ± 0.131	1.515 ± 0.108	0.476
D0.P	26.8 ± 3.713	27.001 ± 4.733	28.52 ± 3.977	0.945
D5.P	72.629 ± 7.584	88.433 ± 14.285	93.973 ± 8.493	0.207
D0.E2	570.29 ± 77.762	744.119 ± 154.377	811.867 ± 83.423	0.154
D5.E2	876.01 ± 100.751	1236.248 ± 141.432	2094.152 ± 128.068	0.000*
Beta hCG	0.835 ± 0.243	46.165 ± 8.462	297.81 ± 38.228	0.000*
E2 /P on D0	34.027 ± 4.957	35.839 ± 8.256	53.256 ± 10.388	0.159
E2/P on D5	15.165 ± 1.61	21.302 ± 3.124	30.248 ± 3.737	0.001*

Values represented as mean ± SD *indicate significant.
 FSH: Follicle stimulating hormone. E2: Estradiol. P: Progesterone.

(22.3%) had non-viable pregnancy (preclinical abortion); and 40 (42.6%) failed to conceive. Patients with clinical pregnancy had significantly greater number of oocytes, fertilised embryos and E2 levels on D5 (Table-1). The E2-P ratio on D0 in the clinical pregnancy group was higher than the rest of the groups, but was not significant, whereas on D5 higher ratio in the clinical pregnancy

group was significant compared to the two other groups. To analyse the prognostic power, the E2-P ratio with respect to clinical pregnancy and pre-clinical abortion, AUC Roc was determined with ROC analysis (Figure-1a and 1b). The AUC suggested a relationship between E2-P ratio on D5 for clinical pregnancy (0.712; 95% CI= 0.605 - 0.815; p<0.001) and for pre-clinical abortion (0.508; 95%

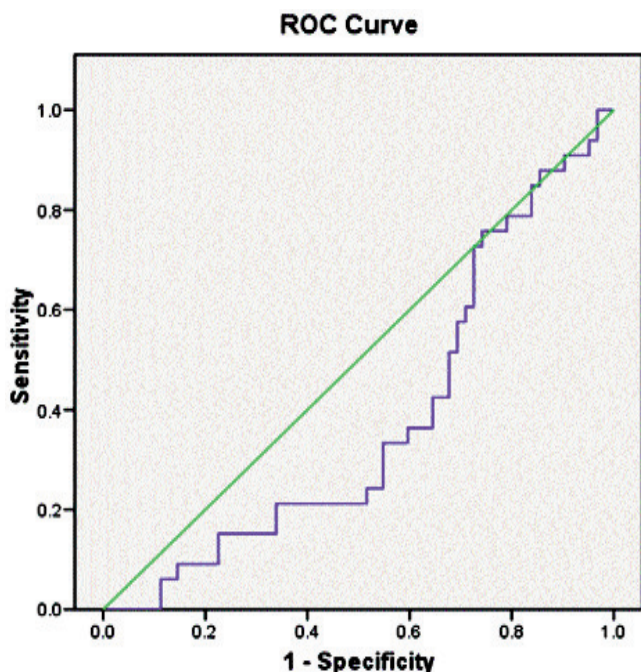


Figure-1a: Receiver operator curve for Estradiol-Progesterone ratio in clinical pregnancy.

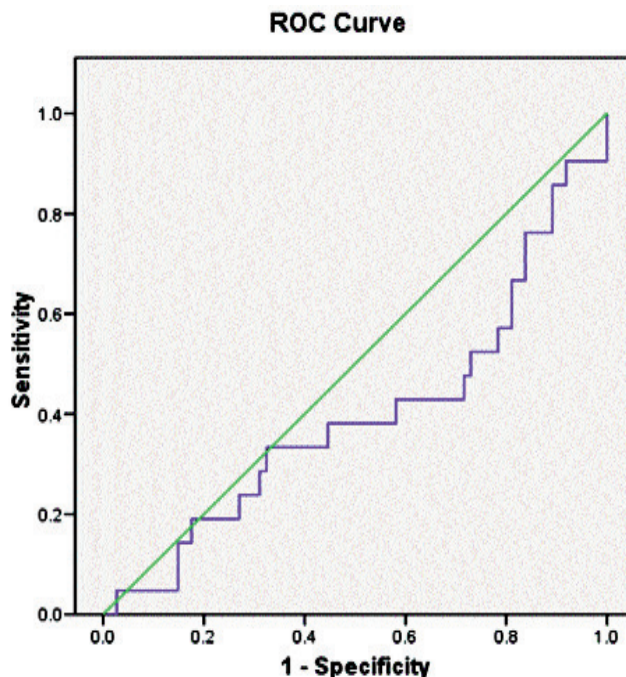


Figure-1b: Receiver operator curve for Estradiol-Progesterone ratio in pre-clinical abortions.

CI= 0.360 -0.657; $p < 0.906$). The AUC (sensitivity/1-specificity) for clinical pregnancy was 0.712 which confirmed the test adequacy.

Discussion

Implantation is achieved by successful intercellular interactions between the developing embryo and receptive endometrium of a hormonally primed uterus in a "window of implantation" at D20-24 of a regular 28-day menstrual cycle.¹³ In the advancing era, recognition, understanding of clinical correlation, hormonal assessment and biochemical evaluation of causes and measures to get better implantation rate may help to bring a paradigm shift in improvement of procedure techniques at ART.

In a normal reproductive cycle, eggs develop in small fluid spaces called follicles in the ovary. As follicles grow, the hormone E2 is produced. When it reaches a certain level, another hormone LH (luteinising hormone) is released. The LH release is the driving force necessary for final maturation and release of the egg or ovulation. The COS regimen employed in ARC is aimed at preventing premature Luteinising Hormone surge¹⁴ either with GnRH agonists or antagonists. There is a controversial discussion about the better regimen choice¹⁵ and clinical advantages of GnRH antagonists over agonists. Results of a study³ however, have proved that selection of either does not affect reproductive outcome. In addition, agonist approach in IVF centres has proved to have greater numbers of oocytes retrieved, better quality embryos, higher pregnancy rates than older protocols¹⁶ and has reduced cancellation rates.¹⁷ Our study used GnRH agonist (long protocol) for suppression of hypothalamic-pituitary ovarian axis and prevention of endogenous LH surge by Injection Deca Peptyl from the mid-luteal phase of the prior cycle until the time of OI.

In treatment procedure of ICSI, after down-regulation with GnRH agonists, high FSH concentrations from the early follicular phase (2nd to 3rd day of periods) were maintained with the help of rFSH in the form of (Puregon) injections till OI. In a normal cycle during the luteo-follicular transition, ovarian steroidogenesis is made possible by up-regulation of gonadotrophin receptors in the ovary.¹⁸ This was made possible in our study by rFSH which continued throughout the follicular phase of the female cycle for multi-follicular growth to recruit a greater number of follicles, improve the antral follicle count and chance of fertilisation for availability of increased number of embryos.

The initiation dose of rFSH (Puregon) calculated with respect to age, basal FSH and number of attempts was titrated by follicular tracking done by TVS from D5 of COS

till OI. The number of ampoules of rFSH used however did not influence the pregnancy outcome. Results are comparable to a study done which proved that rFSH dose of stimulation cycles did not affect pregnancy outcome.³ In present-day IVF procedure, the importance of achieving a good response to COS is obtained by a good quality of oocytes which correlate positively with the ongoing pregnancy rate^{19,20} and, hence, the success of ART. We had similar results in which number of oocytes retrieved by OPU and fertilised had a significant impact on the success of ICSI in terms of clinical pregnancy.

The day and time of OPU was dependant on follicular response to treatment²⁰ extended from D14 to D16 subsequent to stimulation with COS by rFSH. The deviation of OPU from 14th to 16th did not influence clinical pregnancy rate. After OPU, eggs were prepared for ICSI. The number of fertilised embryos in our study correlated with the attainment of clinical pregnancy documented in other studies.^{19,20} The selection of blastocysts in our study was aimed at selecting embryos of superior developmental and highest implantation potential;¹⁴ the blastocysts transfer done is used in numerous laboratories in order to decrease the multiple pregnancy rates and to increase the implantation rate.³ The clinical pregnancy was, however, not influenced by number of blastocysts transfer.

After ET, inadequate uterine receptivity accounts for two-thirds of unsuccessful outcomes²¹ which is attributed to lack of optimal concentrations of hormones, failure of extension of pinopods over the micro villi and asynchrony of time regarding development of blastocysts and pinopods.²² E2 in this regard increases endometrial proliferation, uterine perfusion, and improves the possibility of pregnancy. It has been shown that with "conventional" IVF/ET, optimum implantation potential requires that on the day of the hCG trigger, the endometrium should measure ≥ 9.0 mm and ideally (although less important than thickness) should have a "triple-line appearance". While some viable pregnancies may occur with a lining of 8-9mm, very few will occur when the endometrium measures < 8 mm.²⁰ The endometrial lining at the time of hCG administration correlated with successful outcome ($P=0.001$) in our study.

The ratio of E2-P in conception is important in the sense that P transforms E2-prepared endometrium into a secretory tissue and both create cordial environment for embryo implantation.⁸ There was a rise in E2 and P from D0 to D5 in our study which was significant for E2 compared to P. In our study, blastocysts with highest implantation potential were selected. As a result, implantation and

clinical pregnancy results were attributed mainly to uterine receptivity as depicted by rise in E2-P ratio. The significant high ratio in clinical pregnancies in comparison to pre-clinical abortions and no conception is in agreement with preparation of receptive endometrium required for the continuation of healthy corpus luteum activity, successful implantation and maintenance of pregnancy. Positive outcome of implantation by release of hCG from chorionic villi of the developing embryo was acquired in 57% cases. All these patients had a higher E2-P ratio compared to those who failed to conceive. The females who had a positive pregnancy test and foetal cardiac activity detected by TVS (35%) had significant high E2-P compared to pre-clinical abortions and non-conception groups. The results are comparable to a study³ in which after ICSI, higher E2-P was reflected in conception cycles (pre-clinical abortions and clinical pregnancy) compared to non-conception cycles.

The small sample size and a wider confidence interval were the limitations of our study. However, being the first research in Pakistan for the detection of E2-P ratio at the time of ET, the study can help in the prediction of pregnancy outcomes.

Conclusion

The study demonstrated the likelihood of positive results with increased endometrial thickness and total number of oocytes retrieved on the day of egg collection. Both the elements contribute to a high E2-P ratio on the day of ET and support the hypothesis. The implementation of this knowledge can help in the selection of best treatment protocols, leading to maximum conception rates.

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