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RESEARCH ARTICLE

"ROLE OF HORMONES AND CYTOKINES IN CREATING AN ATTRACTIVE ENDOMETRIAL BED FOR FERTILITY - A QUASI STUDY"

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ABSTRACT

Objective: To determine predictors of endometrial thickness required for implantation of embryo after intracytoplasmic sperm injection (ICSI).

Subjects and Methods: It was a quasi experimental design conducted from July 2011 till June 2012 in "Islamabad Clinic Serving Infertile Couples". During treatment protocol, on ovulation induction day (OI), number of oocytes and endometrial thickness was measured by Trans vaginal scan and estimation of serum estradiol (E2), progesterone (P) and interleukin Iβ (IL-I β) levels by enzyme linked Immunosorbent assay. Pregnancy was detected 10 days after blastocyst transfer by beta hCG value of more than 25 mIU/ml. Cut off value of 8 mm endometrial thickness for acquiring clinical pregnancy was declared by receiver operator curve. Univariable and multivariable logistic regression of endometrial thickness was applied.

Results: Females age was 32.1 ± 4.7 years, BMI 24.2 ± 3.7 kg /$m^2$and endometrial thickness 8.6 ± 3.4 mm (mean ± SD). Out of 282 patients, 101 (36%) had positive pregnancy; 95 (94.1%) had thickness ≥ 8mm. Thickness was predicted by a rise in IL-Iβ and E2 (OR=1.017, 95% CI =1.008-1.026, p < 0.001and OR = 1.002, 95% CI = 1.000 – 1.003, p = 0.029) respectively.

Conclusion: The appropriate endometrial thickness was facilitated by coordination of endocrine and immune mechanisms with a high E2 and IL-Iβ that made endometrium amenable and creating it as a suitable bed for embryo implantation.

INTRODUCTION

The pleasure of fulfilling parenthood is a prime human biological need and perhaps the greatest source of enjoyment for the children's parents. This absence of parenthood is like a dry vacuum in one's life and is a heavy load on the chest of those who are unable to have a family.

Assisted reproductive technology (ART) is an established technique used for infertile human couples, which comprises of scientific techniques employed to overcome existing natural barriers to induce fertilization and it assists fertility for the effected couples to achieve parenthood (Rehman etal., 2013 b). Out of various known methods used to attain parenthood, intracytoplasmic sperm injection (ICSI) is an advanced technique used to obtain the desired objective in which microinjection of spermatozoa is done in the ooplasm at right angles to the polar body. (Rehman etal., 2013c, Rehman etal., 2012 d). Therefore, the high cost factor associated with ICSI, repeated visits to a doctor, a low implantation and high success rate of ICSI procedure in our country has led us to the need, to evaluate predictors of success after performing ICSI. (Sharma etal., 2012)

The success of ICSI depends upon multiple factors and amongst them; quality of the embryo and endometrial receptivity are considered to be imperative in this respect. (Weissman etal. , 1999). It has been found that extending the embryo culture till blastocysts stage helps in the selection of embryos with ongoing developmental potential (Graham et al; 2000). Receptivity means, preparation of endometrium for a synchronized dialogue with the encroaching blastocyst under the influence of hormones and cytokines (Enlanshar and Aboul Enin 2004, Eltouky etal., 2008). Endometrial thickness is one of the measures used for estimation of endometrial receptivity. It is supposed to be maximum at time of ovulation induction (OI), when it is determined by Trans vaginal scan (TVS). The optimal thickness of endometrium has documented to be diverse in different populations ranging from of to 7- 8 to 9-10 mm. (Eltouky etal., 2008, Kovasc etal; 2008) Our study results showed, that thickness of 8 mm was favorable for implantation of embryo and hence occurrence of pregnancy in a pilot study done on same patients. (Rehana unpublished data) .

The endometrial thickness obtained is a contribution of follicular release of multiple hormones like: estradiol (E2), progesterone (P), cytokines including Interleukin-Iβ, adhesion molecules like integrins and growth factors. E2 and P. All

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these have vital role in proliferation of endometrial tissue that increases its vascularity and subsequently also improves implantation and overall pregnancy potentials (Eltouky etal., 2008). Cytokines at the same time have emerged to be an important component of a communication network for embryo-maternal interaction. The objective of our present study was to investigate the role of hormones and cytokines in predicting the required thickness of endometrium responsible for inducing as a suitable bed and favoring pregnancy from ICSI technique in infertile human subjects.

Table 1 Baseline cycle characteristics (N = 282)

Variable	Mean ± SD [a]
Maternal age (years)	32.1 ± 4.7 years
Duration of infertility (years)	7.48 ± 3.68
Body mass index	24.2 ± 3.7 kg /m^2
Antral follicle count	15.6 ± 6.8
Duration of stimulation (days)	11.7 ± 3.4
Total dose of gonadotrophin (IU)	2537.3 ± 1067.2
Endometrial thickness (mm)	8.6 ± 3.4
Estradiol level on hCG day (pg/mL)	2516.43 ± 267.93
Progesterone level on hCG day (ng/mL)	1.47 ± 0.74
Interleukin-Iβ on hCG day (pg/mL)	150.76± 57.63
No. of oocyte retrieved	7.91 ±1.32

PATIENTS & METHODS

Quasi experimental design was conducted on 320 infertile couples in an assisted reproductive clinic of Islamabad (Pakistan) from July 2011 till June 2012. Convenience sampling of females was made on the basis of inclusion criteria; age 20- 40 years, duration of infertility more than 2 years, presence of both ovaries , normal menstruation cycle 28 ± 7 days , body mass index (BMI) of 18–30 kg/m2, basal serum levels of Follicle stimulating hormone (FSH) less than 8 I U/ mL, selected for long agonist protocol. Excluded patients were the females who gave history of uterine fibroids and metabolic disorders, cases of secondary infertility and those who had a history of previous failed ICSI attempts. The treatment protocol comprised of following down regulation with gonadotrophin releasing hormone agonist (Gn Rh a), controlled ovarian stimulation (COS), OI, oocyte pick up (OPU), ICSI followed by embryo transfer (ET). On OI day, endometrial thickness was measured by TVS in the midsagittal plane with a 7.5-MHz vaginal probe (Hitachi EUB 525; Hitachi, Tokyo Japan). Measurements were made at the thickest endometrial segment at the endometrial- myometrial interface. (Friedler etal., 1996).

Samples of peak E2, P and IL-1β were taken by venipuncture, under sterile conditions on the same day and analyzed by Enzyme linked immuno sorbent assay. Pregnancy was diagnosed by a positive blood test after serum beta hCG measurement of specimens obtained by peripheral venipuncture 10 days after ET8 (Toukhy etal; 2008) with non pregnant; β hCG <25 mI U/ml and pregnant with β hCG > 25 mIU/ml.

The cut off thickness of 8mm cut off endometrial thickness was identified by receiver operating curve (ROC) plotted against clinical pregnancy in Med Calc 12.5.0 software.

Statistical Analysis

The data was entered in MS Excel and exported to SPSS (version 15; SPSS Inc., Chicago, IL, USA) for analysis. Clinical characteristics were summarized in terms of frequencies and percentages for qualitative variables, mean ± SD for continuous/quantitative variables. To find out the clinical predictors of pregnancy based on endo-lining, univariable and multivariable logistic regression was run. P value less than 0.05 was considered to depict significant effect.

RESULTS

Out of 320 initiated cycles, 282 females completed the procedure. The cycle characteristics of patients are represented in Table1. Table 2 represents outcomes from Univariable and multivariable logistic regression models of endometrial thickness with hormones and serum IL-Iβ. Crude model depicted significant effect of each individual predictor where increase in P showed decrease chances of being pregnant (OR: 0.310, 95% CI = 0.212 – 0.452, p < 0.001). The other parameters showed that their elevated values caused significant rise of likelihood of being pregnant. Multivariable logistic model declared that controlling the effect of hormones, IL-Iβ helped in increasing endometrial thickness (OR=1.017, 95% CI =1.008-1.026, p < 0.001). Similarly, E2 on OI day significantly enhanced the chances to being pregnant (OR = 1.002, 95% CI = 1.000 – 1.003, p = 0.029). Number of oocytes and P showed insignificant effect in the presence of estradiol and IL-Iβ.

Figure 1 displays the effect of above significant factors on each other while stratified by pregnancy group based on endo-lining cut-off of 8 mm. It revealed that with the increase of one unit IL-Iβ on OI day, E2 increases up to 3.22 units among non-pregnant females while 1.1 units among pregnant females.

DISCUSSION

Implantation of human embryo is a sequenced and a technique sensitive event of apposition, adhesion and invasion with a well coordinated signal exchange between the blastocyst and receptive endometrium in the window of implantation procedure. The importance of estimation of endometrial receptivity lies in the fact that,sixty percent failures occur due to inadequate uterine receptivity due to insufficient concentrations of hormones and cytokines which result in inadequate harmonization in development of blastocysts and pinopods (Enlanshar and Aboul Enin 2004).

Development of endometrium in a normal cycle which is subject to change with respect to hormone secretion in follicular and luteal phase of ovarian cycle. Rehman etal; 2012a) In the treatment protocol of ICSI patients during COS, follicles growth increase supra physiological E2 concentration which increase endometrial thickness due to proliferation of its glandular and stromal components. The increase in serum E2

Table 2 Predictors of endometrial thickness

Variables	Crude Odds ratio	Adjusted Odds ratio
Progesterone level on OI day	0.310 (0.212 – 0.452)	0.665 (0.356 – 1.244)
Estradiol level on OI day	1.003 (1.002 – 1.004)	1.002 (1.000 – 1.003)
Interleukin-1β on OI day	1.024 (1.016 – 1.032)	1.017 (1.008 – 1.026)
No. of Oocytes	1.158 (1.000 – 1.342)	1.053 (0.837 - 1.324)

level during the follicular phase is associated with an increase in endometrial thickness, (Baerwald and Pierson 2004).The importance of increased endometrial thickness is proved from a study in which, obese and infertile females could not conceive because of low E2 levels and decreased endometrial thickness. (Rehman etal; 2012 a).In our study, E2 turned out to be a predictor of endometrial thickness and higher pregnancy outcome. Average peak E2 of 2516 pg/ml was required to acquire endometrial thickness for implantation of ovum whereas E2 levels greater than 4000 pg/ml were documented by others.(Kara etal; 2012). It has recently been documented that Estradiol valerate improved thickness of less than 8mm in patients induced on clomophine citrate therapy. (Satirapod etal; 2014). There are evidences that the administration of estrogen can stimulate the proliferation of endometrium, up regulate the progesterone receptor for continuation of pregnancy. Several studies also report the detrimental effect of elevated E2 on endometrial receptivity.((Enlanshar and Aboul Enin 2004). P is the luteal phase hormone that maintains secretory phase of endometrium by production of specific proteins and growth of receptors.[Bosch etal., 2010] The elevated P level however can accelerate endometrial maturation before time leading to impaired endometrial receptivity (Enlanshar and Aboul Enin 2004). Premature luteinization (PL) is the term used for rise in serum P level on OI day and refers to a range of 0.8 to 2 ng/ml. It was proposed by Melo et al that PL impairs endometrial receptivity more than the oocyte quality in oocyte donation program (Melo et al; 2006). It was also noticed that patients who exhibited elevated P levels at the time of OI had decreased endometrial thickness and reduced pregnancy success rates. This impairment of endometrial thickness reflected by decrease in pregnancy in our research has been laid down and supported by a number of workers (Anderson et al; 2006, Melo et al; 2006)

IL-Iβ proved to enhance all reproductive outcomes including implantation and clinical pregnancy by making endometrium more receptive to implantation with increased thickness of endometrium and expression of adhesive proteins. (Guzeloglu-Kayisli etal; 2007 b) The peak IL-1β on the day of hCG administration was found to be high in Group II that narrates association of endocrine and immune mechanisms for attainment of pregnancy in conception cycles. The results were similar to except that IL-1b was measured 36 and 72 hours after hCG injection, while in our study we measured it on hCG day. IL-1β showed a positive significant correlation with, endometrial thickness. (Watanabe etal.; 1994).

It has been observed that positive results of embryo implantation rely on quality of transferred embryos and endometrial receptivity (Weissman etal., 1999). In our study, since we used blastocyst of highest implantation potential hence endometrial receptivity was the most important predictor of success after ICSI. The estimation of endometrial thickness on OI day thus can give an indirect evidence of E2 and IL-1β and therfore outcome results in terms of raised βhCG. This study and its findings are limited in the sense that, it has not evaluated endometrial pattern or echogenecity which are more reliable methods of estimation of endometrial receptivity. (Rashidi etal; 2005, Franchin etal; 2001) and furthermore, it does not cover the endometrial sampling which is now used in patients of repeated implantation failure (Ruiz etal; 2013).

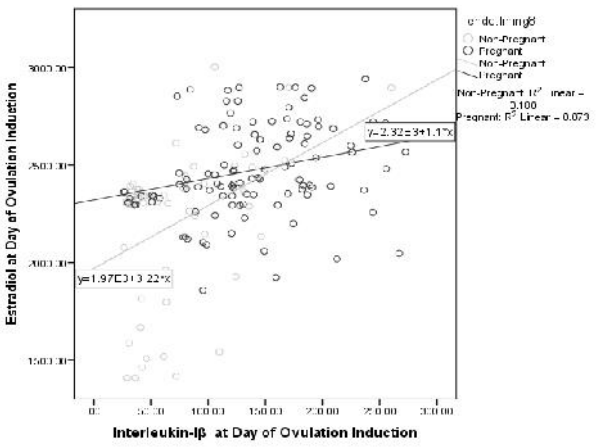

Fig. 1 Scatter Plot of Estradiol and Interleukin-Iβ at the Day of Ovulation Induction Stratified by Pregnancy Group based on Endo-Lining Cut-off of 8 mm

CONCLUSION

In patients of ICSI, endometrial receptivity measured in terms of its thickness by TVS on OI day depends on optimal amounts of secretion of hormones and cytokines from their cellular bed.

Recommendations

Endometrial thickness should be estimated on OI day in all patients undergoing ICSI treatment cycles.

Variables measured on the day of ovulation induction (OI) entered

Logistic regression applied on endometrial thickness and its determinants

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