RESEARCH ARTICLE

INTERPLAY OF ENDOCRINE AND IMMUNE MECHANISMS FOR ENDOMETRIAL RECEPTIVITY AFTER INTRACYTOPLASMIC SPERM INJECTION

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ABSTRACT

Objective

To observe relationship of optimal endometrial thickness with change in interleukin (IL-I), estradiol and progesterone from follicular to luteal phase in females of intra cytoplasmic sperm injections

Subjects and Methods

A cross sectional survey of564females of ICSI was carried out from July 2011 to November2013.Receiver operating curve identified 8 mm endometrial thickness for implantation and clinical pregnancy in these females. They were categorized into group I and II on the basis of endometrial thickness less than and greater than cut off value (8 mm) respectively. Estimation of hormones estradiol (E2) and Progesterone (P) with interleukin I- (IL-I)was done in follicular and luteal phase by enzyme linked Immunosorbent assay.

Results

Study comprised of 232 (41%)and 332 (59%) females in Group I and II respectively. The P levels were significantly lowin group II (pregnant females with optimal endometrial thickness) in both phases (p<0.0001). On the other hand, E2 was less in follicular phase and more in luteal phase of group II when compared to non-pregnant females (p<0.0001). IL- I was greater infollicular and less in luteal phases of group II females (p<0.0001).

Conclusion

In females for optimal endometrial thickness for implantation, increased E2production in luteal with respect to follicular phase and perseverance of low P levels was observed. A significant high ratio of IL-I from follicular to luteal phase in these females highlights the role of immune mechanisms together with changes in hormonal parameters for endometrial receptivity required for implantation.

INTRODUCTION

In recent years, number of infertile couples have dramatically enhanced with an aggravation of medial, social, psychological and economic burdens on developing countries.(Rijal *et al.*,2011) In vitro fertilization (IVF) or Intra cytoplasm sperm injection (ICSI) are established practices of "assisted reproductive clinics (ARC)" where conception does not occur by normal and routine infertility treatment procedures. These expensive treatment procedures unfortunately have a restricted 30% success rate; therefore ARCs make their level best to carry out all such measures that attempt to improve success rates. (Rehman *et al.*, 2012 a, Rehman *et al.*, 2013)

For conception, window of implantation is defined as the time period during which uterine environment supports blastocyst acceptance followed by its attachment to the conducive endo metrium.(Elnashar and Aboul-Enin2004) Endometrium is a vivacious tissue that proliferates, differentiates and sheds in response to changes in the levels of hormones and cytokines during the phases of menstrual cycle.(Rehman *et al.*,2014 b) Endometrial thickness may vary from 0.5mm to 7 mm in the follicular phase that may become as profuse as 15 mm, maintaining this during the luteal phase of the cycle. This thicknessis obtained by release of multiple hormones like: estradiol (E2), progesterone (P), cytokines including Interleukin-I , adhesion molecules like integrins and growth

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factors released infollicular and luteal phase of cycle.(Rehman et al.,2014a)

The role of E2in this regardis to synchronize endometrium for the reception of blastocyst implantation by hypertrophy and hyperplasia with development of P receptors.(Rehman et al.,2014 c) The cytokines secreted by the endometrium and normal ovarian tissue can result in a pro or anti-inflammatory environment in this window of implantation. Interleukins (IL) likeIL-1 , IL-6, and IL-18 are identified as key mediators of inflammation and arbitrate many pathways of the normal immune response. (Bedaiwy et al., 2007, Vujisic et al., 2006) Out of pro-inflammatory cytokine IL 1 tends to activate certain mechanisms which enhance the angiogenesis of the endometrium thus increasing its size and thickness (Carino et al., 2008)

We wanted to recognize endocrine immune interplay required for the optimal endometrial thickness and hence implantation in patients after ICSI. The study thus, aimed to compare cytokine (IL-I) and hormonal profiles during the follicular and luteal phases of assisted reproductive cycles in groups stratified on the basis of endometrial thickness.

MATERIAL AND METHODS

The cross sectional study was conducted on 640infertile couples in an assisted reproductive clinic of Islamabad, Pakistan, from July 2011 to November 2013. Convenience sampling of consented females was done and treatment protocol was followed as described in figure1. Ethical approval of the present study was acquired from "Islamabad Clinic Serving Infertile Couples".

Treatment protocol of the subjects is described in Figure1. On the day of ovulation induction (OI) endometrial thickness was measured in mid sagittal plane by two-dimensional ultrasound with a 7.5-MHz vaginal probe (Hitachi EUB 525; Hitachi, Tokyo Japan) (Friedler et al., 1996) and venous sample was collected for E2, P and IL-I .

After oocyte pick up (OPU), ICSI of mature oocytes was done and embryos were incubated till differentiation into blastocysts. Embryo transfer (ET) was performed 5±1 days after OPU. Pregnancy was declared by a positive pregnancy test by serum beta hCG measurement of specimens done 10 days after ET.(El-Toukhy et al.,2008)Patients were categorized into non pregnant with endometrial thickness < 8 mm (Group I) and pregnant endometrial thickness > 8 mm (Group II) on the basis of cut-off values established after performing receiver operating curve analysis.

Statistical Analysis

The data was entered in MS Excel and exported to SPSS version 20 for the analysis. Variables were summarized in terms of frequencies and percentages for categorical variables and with mean ± SD for continuous variables. Normality of continuous variables within each group was checked by Shapiro-Wilk test and identified that all variables except Estradiol ratio were skewed as p values were less than 0.05. Therefore, mann-whitney U test was run to assess difference between demographic and clinical factors between group I and II females while independent samples t-test was used to compare mean values of estradiol ratio. Significant difference was declared when the test produced p value less than 0.053.

RESULTS

Our study females in Group I (non-pregnant; endometrial thickness < 8 mm) comprised of 232, (41%) and 322(59%) in group II (endometrial thickness >8 mm). The comparison of demographics, hormones and cytokines on the basis of cut off thickness of endometrial thickness 8mm is shown in table 1and 2. Age and BMI were similar in both groups (table 1). Duration of infertility was slightly more among non-pregnant female (group I). Duration of stimulation was marginally more in group II. Number of oocytes in this group with greater endometrial thickness were nearby significantly larger in quantity as compare to their non-pregnant counterpart (P = 0.051).

Table 1; Biophysical parameters of study subjects

Variables	Group I Endometrial thickness < 8mm (n=116)	Group II Endometrial thickness 8mm (n=166)	P Values
Age (years)	32.24± 4.55	32.02 ± 4.74	0.718
Body mass index(kg/m^2)	23.94 ± 3.65	24.46 ± 3.71	0.268
Duration of infertility (years)	7.56± 3.98	6.79 ± 3.77	0.099
Duration of stimulation (day of egg collection)	14.20± 0.92	14.43 ± 1.00	0.061
Oocytes	7.46±1.61	7.86±1.70	0.053

Table 2 Comparison of hormones and IL- I-

Endo Categories	Group I	Group II	P Values
Progesterone(ng/ml)*	1.82 ±0.69	1.24 ± 0.68	<0.0001
Progesterone(ng/ml)	179.60 ± 34.17	127.33 ± 55.39	<0.0001
Follicular to Luteal Progesterone ratio	0.01 ± 0.004	0.01 ± 0.01	0.221
Estradiol (pg/ml)*	2494.35±336.56	2412.85± 229.68	<0.0001
Estradiol (pg/ml)	904.77±142.56	1023.55± 157.76	<0.0001
Follicular to Luteal Estradiol ratio	2.46±0.44	2.40±0.35	0.191
Interleukin I- pg/ml)*	71.79±49.61	139.42± 57.42	<0.0001
Interleukin I- pg/ml)	60.45 ± 24.07	46.13± 15.67	<0.0001
Follicular to Luteal Interleukin I- ratio	1.10±0.86	3.53±2.45	<0.0001

*Follicular phase,   Luteal phase

While comparing hormones and cytokines in follicular and luteal phase (table 2) significant differences of these parameters in both groups (P values < 0.0001) were noticed. The P levels were significantly lower in group II in both follicular and luteal phase. On the other hand, E2 and IL-I showed variant outcomes on these two days. E2 was less in follicular phase of group II as compared to their non-pregnant similitudes while in luteal phase; E2 was more in this group of females. IL- I was greater in group II in follicular phase whereas reverse results were observed in luteal phase. When putting into account the ratio of P, E2 and IL-I , it was observed that ratios of progesterone and estradiol were not significantly different in two groups, however, the ratio of interleukin I- was significantly low in non-pregnant females.

DISCUSSION

Successful implantation is credited to accessibility of top quality embryos and receptive endometrium sanctioned to optimal levels of hormones precisely E2 and P and a number of cytokines. (Rehman *et al*., 2014 c) Failure of implantation remains the most important limiting factor determining IVF/ICSI success ratesand point to be taken care of in the ARC. The exact role of autocrine, paracrine, and endocrine factors responsible for endometrial receptivity thus needs to be explored to overcome barriers of implantation.

It is hypothesized that implantation failure following ICSI treatment may be caused by impairments in both endocrine and immune systems. As far as hormones are concerned, favorable effect of high follicular E2 on number and maturity of oocytes has been observed by a number of researchers.(Rehman *et al*.,2012 a)In the present study high follicular E2 levels helped in acquiring required endometrial thickness. A correlationof endometrial thickness with serum E2 levels in normal and COS cycles, and its role in predicting endometrial maturation has been documented.(Elnashar and Aboul-Enin2004,Rehman *et al*.,2012 b)Our study is supported by Simon etal in which reduced implantation rate with E2 levels above 2500 pg/ml was noticed without any impairment of embryo quality.(Simon *et al*., 1995)Nevertheless in a comparative study of ICSI and donated oocytes patients, no negative impact on embryo quality or pregnancy rate could be detected in patients with supra physiological E2 levels.(Levi *et al*.,2001)

Results have established that increased E2 in the window of implantation (which is in the mid luteal phase) enhances the chances of conception.(Rehman *et al*.,2014 b) On the basis of present study we came to know that females who exhibited optimal endometrial thickness continued to have high E2 levels in midluteal phase as is required for functional corpus luteum formation for conception. These resultsof high luteal E 2 with conception are similar to Ganesh *et al* except that they measured E2 levels in the late luteal phase and did not find its association with endometrial thickness.(Ganesh *et al*.,2009)

The relationship between serum P level and outcome of ET after IVF and ICSI has been controversial for several decades.(Rehman *et al*.,2012 b, Bosch *et al*.,2010) Some of the researchers reported that the serum P elevation changes the implantation window rather than embryo quality. (Melo *et al*.,

2006) The findings of our study revealed higher P levels in follicular and luteal phasein-group I, which may explain for decreased endometrial thickness and hence un-successful cycle in these individuals. The results are supported by Valbuena *et al* in which high P levels were shown to reduce both endometrial receptivity and embryo quality. (Valbuena *et al*., 2001) It is already reported that reduced P levels with high E2 and P ratio helped in implantation of fertilized oocyte.(Rehman *et al*.,2014 a)

We found lower values of IL-1 in group I which indicates impairment of folliculogenesis that might have occurred and contributed to decreased endometrial thickness in these women.(Vassiliadis *et al*.,2005)These results demonstrate role of IL-1 in promoting follicular growth, steroidogenesis, recruitment and activation of leukocytes necessary for ovulation and tissue remodeling during ovulation.(Buscher *et al*., 1999)The role of IL-1 in oocyte maturation and fertilization in patients of ICSI is documented by a number of workers.(Vujisic *et al*., 2006) Studies have shown similar effects of cytokines such as IL-18, with oocyte fertilization and embryo development and successful IVF-induced pregnancy. (Sarapik *et al*., 2012)The highlevels of IL-I in patients of group I expressed its role in luteinization, and luteolysismay have influenced endometrial receptivity thus causing fertility failure.

Measurement of endometrial thickness on OI day by TVS isno doubt a reliable indicator of hormones and cytokines in the follicular phase.(Rehman *et al*., 2014, Bonetti *et al*., 2010) We observed that pregnant females (group II) acquired this by more E2 and IL-I production with concomitant fall in both during the luteal phase. The P levelin this regards is subject to bias due to use of P pessaries started on OI day. Our study although is limited in terms of small sample size confounding factors like more oocytes in group II yet this is the first study which highlights role of a high ratio of IL-I in follicular to luteal phase for optimal endometrial thickness. The research strengthens the measurement of endometrial thickness to reflect interplay of hormones and cytokines in follicular and luteal phases of ovarian cycle which are required for preparation of endometrial bed for implantation.

CONCLUSION

Endometrial thickness estimation can provide an information about role of cytokine (IL-I), E2 and Plevels in follicular and mid luteal phase of ovarian cycle. We observed that females who conceived with optimal endometrial thickness (8 mm) had a high IL-I and low E2, P levels in follicular phase. During the luteal phase,E2 production increased with a significant reduction in P and IL-I levels.

A significant high ratio of IL-I from follicular to luteal phase and increase in E2 production in luteal phase emphasizes the role of immune mechanisms and hormonal parameters for endometrial receptivity required for implantation. The endometrial thickness measured on the day of ovulation induction can thus give information about hormone and cytokine interplay required for implantation.

Figure I
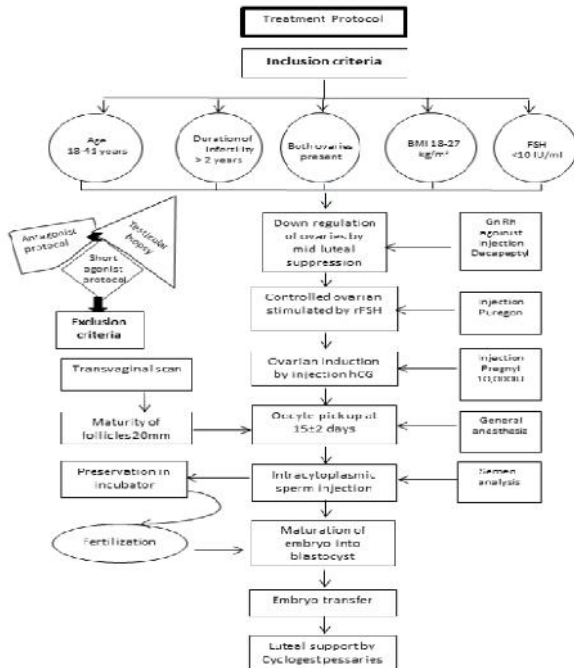
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