Original Article

Role of progesterone in human embryo implantation

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ABSTRACT

Objective

To identify causes of embryo implantation failure after intra cytoplasmic sperm injection (ICSI); by correlation of positive pregnancy outcome with age, number and grading of embryos, day of egg collection, endometrial lining and progesterone levels on the day of egg collection and embryo transfer.

Materials and Methods

This descriptive case study was conducted on 40 couples of Assisted Reproductive Clinic in Islamabad from June till August 2011. Down regulation of females aged 18-35 with Gonadotrophin releasing hormone agonist was followed by calculated stimulation with gonadotrophins (Injection Puregon). Fertilized eggs were graded, blastocysts transferred on day 13, 14 and 15 of treatment cycle on the basis of follicular maturation and endometrial lining measured by trans vaginal scan. Serum progesterone was measured on the day of egg collection and embryo transfer.
Results

Rise of progesterone in secretory phase of endometrial cycle more so on the day embryo transfer (ET) was associated with positive pregnancy outcome. However, female age, number and grading of embryos, day of ET, endometrial lining and other biochemical parameters had no significant effects.

Conclusion

Estimation of serum progesterone on the day of ET is highly specific and sensitive test for prediction of positive pregnancy outcome as detected by rise in β hCG (Human Chorionic gonado trophin). (Rawal Med J 2012;37:194-198).

Key Words

Intra cytoplasmic sperm injection, embryo transfer, Egg collection, Progesterone, β hCG.

INTRODUCTION

Embryo implantation is the consequence of successful intercellular interactions between developing embryo and receptive endometrium of a hormonally primed uterus in a “window of implantation” at day 20-24 of a regular 28 days menstrual cycle.¹ A healthy couple by and large conceives by normal physiological mechanisms during a period of six months to two years after marriage² with regularity of well-timed unprotected intercourse. In vitro fertilization (IVF) or Intra cytoplasm sperm injection (ICSI) is highly developed practice of “Assisted reproductive treatment” procedures where pregnancy fails to occur by normal and routine infertility treatment procedures.

The whole process of IVF and ICSI, from initiation of ovulation to embryo transfer in human beings, is accomplished in a period of four to six weeks. In these procedures, injectable gonadotrophins are given for follicular growth after which mature eggs are
retrieved under general anesthesia by a double lumen oocyte aspiration needle set. ICSI involves the insertion of single selected spermatozoon directly into prepared oocytes, while in IVF, sperms and eggs are kept together in culture medium. Assessment of fertilization, cleavage of embryos and its grading is monitored regularly under microscope. Embryos are transferred approximately five days after fertilization by using Sims-Wallace Embryo Replacement catheter. The hormonal support in luteal phase is provided by progesterone; administered through oral, intramuscular or intravaginal routes (pessaries).

The process of embryo implantation occurs as a result of well orchestrated series of events, 5-7 days after embryo transfer (window of implantation) and success of procedure is dependent on embryonic development in vitro as well as endometrial preparation. Positive outcome is detected approximately fourteen days after embryo transfer by increase in concentration of hCG released by the chorionic villi of developing embryo to more than 25mg/d.

A negative pregnancy test means failure of implantation, which is the most common and disheartening outcome of treatment procedures in assisted reproductive clinics. The breakdown responsibility can be distributed to a wide array of factors contributed by lack of coordination of maternal and fetal interfaces like maternal age, ovarian reservoir, down regulation protocol, ovarian stimulation with gonadotrophins, day of egg collection, endometrial lining, grading of embryos, biochemical parameters and hormonal assessment. Progesterone, a steroid hormone secreted by corpus luteum during the ovarian cycle, is vital for preparation of endometrium for implantation of embryo, successful conception and continuation of pregnancy. Increased blood concentrations of progesterone in the mid-postovulatory phase of the menstrual cycle above the threshold values were associated with
increased live birth rates in an ovulatory women undergoing ovulation induction with gonadotrophin hormones.\textsuperscript{6} The objective of our study was to determine relationship of implantation failure with age, number of oocytes, number of blastocysts transferred, endometrial lining at the time of transfer, day of egg collection (14\textsuperscript{th}, 15\textsuperscript{th} or 16\textsuperscript{th} day) and the relation of maternal serum progesterone at the day of egg retrieval and embryo transfer with the implantation rate.

**MATERIALS AND METHODS**

It was a descriptive case series conducted in Islamabad Clinic Serving Infertile Couples, after approval from their Ethical Review Board from June till August 2011. Forty couples who consented were enrolled for the study. Non-probability (purposive sampling) technique was applied. ICSI cases with a sperm count at least 0.5 millions/ml with 10\% normal sperm morphology according to strict Kruger’s criteria were included while cases of sperm collection from testicular biopsy and females with history of endometriosis, polycystic ovarian syndrome and short term down regulation protocol were excluded. All females were down regulated with daily injection Deca Peptyl (Gonadotripin releasing hormone agonist) from Day 21 of previous cycle followed by follicle stimulation by gonadotrophins (Only Inj Puregon) for fourteen days; dose was titrated with respect to basal follicular stimulating hormone (FSH) and follicular monitoring by transvaginal ultrasound was done 3-4 days after the commencement of the ovarian stimulation till maturity of maximum follicles to 20 mm.

Before egg collection, complete blood count, serum urea creatinine, random blood sugar, serum electrolytes and initial progesterone estimation was done. Mature oocytes (20mm) were retrieved 36 hours after hCG injection by vaginal ultrasound probe with 16G adapter
and double lumen oocyte aspiration needle on 14\textsuperscript{th}, 15\textsuperscript{th} or 16\textsuperscript{th} day of stimulation. 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 criteria and film was prepared by Silselect gradient. Micro injections of spermatozoa were performed at right angles to the position of polar body when viewed through the microscope. After confirmation of fertilization and cleavage (18 to 24 hours after egg collection), grading of embryos from 1-5 was done and blastocysts transferred on Day 5 by Sims-Wallace Embryo Replacement Catheter under ultrasound guidance.\textsuperscript{7} Serum progesterone was repeated by Enzyme Linked Immuno sorbent Assay technique on the day of ET. Luteal support of included females (after egg collection) was maintained by progesterone vaginal pessaries (Cyclogest 400 mg) twice a day. ß hCG was assessed at the end of treatment approximately 14 days after ET as the outcome marker.

\textbf{Statistical Analysis:} Statistical comparison was performed by using student t-test for quantitative variables and Chi-square test applied for comparison of qualitative variables with pregnancy. Receiving operating curve (ROC) curve was used for cut of value of progesterone level at day1, day5 for sensitivity, specificity, positive and negative predictive value. A p<0.05 was be considered significant. The data analysis was done by SPSS v 11.0.

\textbf{RESULTS}

A total of 40 females with infertility and age rang 18-35 years were included in the study. Out of these, 13 (32\%) achieved pregnancy after ICSI. No statistically significant association was found with age, type of blastocysts (early, expanding) and day of egg collection (p>0.05).
Table 1. Comparison of Progesterone Day 1, Day5, Endometrial lining and oocytes with pregnancy.

<table>
<thead>
<tr>
<th></th>
<th>Pregnancy</th>
<th>Number</th>
<th>Mean</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone at D1</td>
<td>No</td>
<td>27</td>
<td>18.2</td>
<td>2.19</td>
<td>0.001 *</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>13</td>
<td>37.4</td>
<td>6.43</td>
<td></td>
</tr>
<tr>
<td>Progesterone at D5</td>
<td>No</td>
<td>27</td>
<td>20.4</td>
<td>3.47</td>
<td></td>
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<tr>
<td></td>
<td>Yes</td>
<td>13</td>
<td>401.6</td>
<td>118.21</td>
<td>0.001 *</td>
</tr>
<tr>
<td>Endometrial lining</td>
<td>No</td>
<td>27</td>
<td>9.4</td>
<td>0.51</td>
<td>0.424</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>13</td>
<td>8.7</td>
<td>0.51</td>
<td></td>
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<tr>
<td>Oocytes</td>
<td>No</td>
<td>27</td>
<td>16.9</td>
<td>1.50</td>
<td></td>
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<tr>
<td></td>
<td>Yes</td>
<td>13</td>
<td>18.2</td>
<td>1.57</td>
<td>0.573</td>
</tr>
</tbody>
</table>

Mean progesterone level at day 1 were significantly high in pregnant female 37.4±6.43 as compared to non pregnant 18.2±2.19 (p=0.001). Similarly, mean progesterone level on day 5 were significantly high in pregnant female 401.6±118.21 as compared to non pregnant 20.4±3.47 (p=0.001) (Fig1). No significant difference was found in endometrial lining and oocytes in pregnant and non pregnant female (p>0.05) (Table 1).
There was no significant difference observed in biochemical parameters except eosinophils count which was significantly high in patients who failed to conceive by ICSI. Fig.2 shows the results of area under the ROC curve analysis of progesterone level of day 1 and day 5 which showed that the diagnosis accuracy of progesterone level of day 5 was better than day 1. Cutoff levels providing desired sensitivities and specificities can be deduced from ROC curves. When the point on a curve closest to the upper left corner of the box corresponding to 100% sensitivity and 100% specificity (0 false positivity) was selected, it resulted in cutoff levels of Progesterone on day 1 and day 5 as >22.8 and >67.0 respectively for pregnancy.
Fig 2. Receiver operating characteristics (ROC) curves of Progesterone day 1 and day 5 for differentiating between pregnancy positive and negative females.

The sensitivity and specificity corresponding to these cut off were 84.6%, 70.4% for progesterone level at day 1 and both 100% for progesterone level at day 5 respectively.

**DISCUSSION**

There is a long list of maternal and fetal factors that could be blamed for 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. Ovarian reserve is the potential of ovaries to inherit, retain and recruit
eggs for ovulation. Poor responder is a term used for women who either has no or diminutive ovarian reserves due to genetic predisposition, late marriages, advanced age exposure to radiations, insecticides, lead, toxic chemicals, smoking alcohol and infections etc. Our study showed no significant relationship of implantation failure with female age.

During follicular recruitment, the oocyte acquires maturity by increase in ribonucleic acid synthesis for reorganization of cytoplasmic organelles, secretion of zona pellucida for support, strength and protection. These modifications are controlled intensively by nutrients, growth regulators, optimal concentration and interplay of various hormones precisely FSH, Luteinizing Hormone, Estrogens and Progesterone. Oocytes with a high viscosity of cytoplasm assessed by persistence of injection funnel after withdrawal of ICSI pipette is associated with delayed cleavage and decreased pregnancy rates. After fertilization, embryo is graded on a scale 1-5 on the basis of increased multiplication of blastomeres and decreased quantity of cytoplasmic fragmentation. After ET, inadequate uterine receptivity accounts for two thirds of unsuccessful outcomes which is attributed to lack of optimal concentrations of hormones, failure of extension of pinopods over the microvilli and asynchrony of time regarding development of blastocysts and pinopods. There was no relation with endometrial lining at the time of transfer and day of egg collection (14th, 15th or 16th day).

Progesterone is a hormone of secretory phase that regulates mast cell maturation and degranulation to produce cytokines and growth factors resulting in increase of uterine receptivity. Therefore, an insufficient progesterone concentration or an inadequate response to progesterone may turn out to be cause of infertility and pregnancy loss. Both
these effects are due to a defective luteal phase (time between ovulation and menstruation beginning) that should be sufficient to allow a fertilized egg to implant and the lining to grow and help in adhesion of blastocysts. In our study, there was significant correlation of pregnancy with progesterone on day of egg collection and positive predictive value on the day of embryo transfer with the pregnancy outcome. In another study, serum progesterone levels of >1.5 ng/ml was associated with lower ongoing pregnancy rates following IVF/ICSI cycles irrespective of the Gonadotrophin Releasing hormone analogue used for pituitary down-regulation.\(^{14}\)

Women with endometriosis, and polycystic ovarian syndrome, have an altered progesterone response, which may explain some of the clinical features of these disorders and supports the hypothesis that progesterone resistance underlies some cases of human reproductive failure.\(^{15}\) In a study, after hCG administration, there was a higher trend of pregnancy with increased progesterone serum levels.\(^{16}\) In our study, serum progesterone at the time of egg collection was raised significantly and it was much more raised on the day of ET on the 5\(^{th}\) day after egg collection. The correlation of rise in Progesterone for implantation of human embryo explains its role in preparation of endometrium for blastocysts invasion, successful conception and continuation of pregnancy.

Limitations of our study are a small sample size because of strict inclusion criteria and lack of comparison of Progesterone levels with Estradiol. Yet being the first report from Pakistan can help in prediction of pregnancy outcome as well as supplementation of progesterone by oral and parenteral administration in definite cases. The outcome will be a sense of satisfaction as well as achievement for those who really care for the agonizing pains of infertile couples.
CONCLUSION

Positive pregnancy outcome can be predicted by rise in serum progesterone at the time of ET in ICSI couples.

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REFERENCES


Implantation of embryo was facilitated by better oocyte parameters, oocyte maturity, fertilisation and its cleavage in females who exhibited endometrial thickness above the cut-off value. Assessment of the role that either progesterone insufficiency or inadequate progesterone response plays in human reproductive failure has been difficult to assess because serum progesterone concentrations fluctuate markedly, limiting the ability to characterize sufficiency of progesterone, and there are no highly reliable markers of endometrial function available. Functional embryo at the blastocyst stage and a synchronized dialog between maternal and embryonic tissues [5]. The human endometrium undergoes a complex series of organized proliferative and secretory changes in each menstrual cycle and exhibits only a short period of receptivity, known as the ‘implantation window’ [6]. Endometrial receptivity during the implantation window requires a close cooperation of an extremely large number of. Progesterone is also able to reduce the contractility of the myometrium at the time of the implantation [13]. The crucial role of the corpus luteum in human reproduction in the maintenance of early pregnancy is demonstrated by the harmful effects of a lutectomy during the first weeks of a pregnancy. Physiologic Events of Embryo Implantation and Decidualization in Human and Non-Human Primates. by Maria Ariadna Ochoa-Bernal. The imposition of progesterone over estrogen defines the ‘window of implantation’ (days 20–24) during the secretory phase [1,10,11]. 2.3. The Window of Uterine Receptivity. Successful embryo implantation requires a functional communication between a blastocyst and a receptive endometrium during a brief period of time known as the window of implantation [12,13]. The process of implantation in humans and primates involves a coordinated sequence of events that are critical for the establishment of pregnancy. There are many mediators under the control of ovarian hormones that are crucial during endometrial receptivity.