

# Preimplantation and postimplantation therapy for the treatment of reproductive failure

**Pratap Kumar,  
Siddharth Mahajan**

Department of Obstetrics and Gynecology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India

**Address for correspondence:**

Dr. Pratap Kumar,  
Department of Obstetrics and Gynecology, Kasturba Medical College, Manipal University, Manipal - 576 104, Karnataka, India.  
E-mail: pratap.kumar@manipal.edu

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## ABSTRACT

Treatment of patients with recurrent pregnancy losses and recurrent implantation failure can be instituted only when the underlying etiology is determined. Embryo-secreted preimplantation factor (PIF) is essential for implantation and adequate trophoblastic invasion. Deficiency of PIF affects the outcome of the pregnancy leading to recurrent pregnancy losses. Synthetic PIF modulates the outcome of the pregnancy decreasing the incidence of recurrent implantation failure and recurrent pregnancy losses. In this article a thorough search is done regarding the data published for diagnoses of reproductive failure and its treatment. The effect of immunoglobulin (Ig), intralipid, heparin, aspirin, progesterone, estrogen, and granulocyte colony stimulating factor (G-CSF) is taken into consideration. Heparin, aspirin, and progesterone have successfully shown to decrease the incidence of recurrent pregnancy losses; whereas G-CSF, intralipids, estrogen, and Igs have shown success in the treatment of the recurrent implantation failure and recurrent pregnancy failure. The pregnancies treated with Igs and intralipids showed equal outcome when evaluated and compared. The place of intralipid in reducing natural killer (NK) cells has been discussed.

**KEY WORDS:** Implantation, medication, success

## INTRODUCTION

The exact etiologies for the recurrent pregnancy losses have not been clearly explicated, and time and again remain undefined.<sup>[1]</sup> Recurrent pregnancies and implantation failure is physically as well as emotionally distressing for the patients. Several studies implicated numerous etiologies for the recurrent pregnancy and implantation failure out of which immunological disorders play a significant role.<sup>[2]</sup> One of the most common immunological causes for reproductive failure is elevated level of natural killer (NK) cell activity<sup>[3]</sup> and antiphospholipid antibodies.<sup>[4]</sup> Reproductive failure is unexpectedly common with the fertilized eggs not leading to live births.<sup>[5]</sup> These reproductive failures can be categorized depending on the time they occur during pregnancy into preimplantation, peri-implantation, and postimplantation. Clinically women experiencing preimplantation failures presented with unexplainable infertility. Women with peri-implantation failure

after *in vitro* fertilization (IVF) and embryo transfer (ET) presented with recurrent implantation failure. Recurrent implantation failure has been defined as unsuccessful conception after three cycles of IVF or ET and it can be due to uterine or embryo factors.<sup>[6]</sup> The American Society for Reproductive Medicine has defined recurrent miscarriages as two or more failed pregnancies.<sup>[7]</sup>

## IS ESTRADIOL AND PROGESTERONE THERAPY BENEFICIAL DURING IVF-ET TREATMENT?

One of the most significant factors for implantation is an appropriate hormonal environment. Adequate hormonal concentration is crucial for the implantation and an excessive dosage may lead to detrimental effects on endometrium making it unsuitable for implantation, and hence resulting in implantation failure.<sup>[8-10]</sup> A study<sup>[11]</sup> done on a subset of patients with recurrent miscarriages, were treated with one of the following therapeutic options following

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IVF-ET treatment; human chorionic gonadotropin (hCG) injection, progesterone, estradiol, gonadotropin releasing hormone agonists, cytokines (e.g., granulocyte colony stimulating factor (G-CSF)). The results of the outcome of each of these patients were taken in to consideration which was then compared. It was found that hCG and progesterone turned out to be the best drugs with excellent outcome; however, hCG is associated with high risk of ovarian hyperstimulation syndrome. Vaginal progesterone is associated with better outcome and least side effects. In various studies, progesterone supplements for women with recurrent miscarriages secondary to corpus luteal insufficiency has been seen to be associated with a wide range of success and is used broadly in clinical practice. A world wide web survey<sup>[12]</sup> including 84 treatment centers across 35 countries with a total of 51,155 IVF cycles/year participated with vaginal, intramuscular, and oral progesterone therapy after implantation during IVF treatment showed increased success in live births with maximum IVF centers using micronized vaginal progesterone as the main modality for administration. In 67% of the cycles, progesterone is continued till 10<sup>th</sup> to 12<sup>th</sup> weeks of gestation. Although the duration of progesterone therapy is still in dispute, a study by Kohls *et al.*, has shown the same outcome when micronized progesterone therapy discontinued at 5<sup>th</sup> or 8<sup>th</sup> week of gestation.<sup>[13]</sup>

In poorly responding patients, luteal estradiol pretreatment has also shown improvement. The improvement in outcome is seen when estradiol is given before stimulation protocol, as compared to standard protocol which was shown in a study published by Chang and Wu.<sup>[14]</sup> Luteal estradiol pretreatment protocol given to 450 IVF patients and standard gonadotropin-releasing hormone (GnRH) antagonist protocol given to 606 patients reflected that the stimulation duration was significantly higher with estradiol pretreatment and the number of mature oocytes attained was significantly greater in number. Whereas, another study by Davar *et al.*, reflected the same results, but the number of oocytes retrieved were similar in number.<sup>[15]</sup> Estrogen supplementation has also shown immense success in patients with recurrent implantation failure due to thin and unresponsive endometrium. Shen *et al.*, have successfully proven this by the usage of extended estrogen supplementation on patients with implantation failure before the standard controlled ovarian hyperstimulation with accomplishment of uneventful pregnancies.<sup>[16]</sup>

Chang *et al.*, have shown the effect of luteal phase estrogen in poor responders in IVF.<sup>[17]</sup> In the study, 155 patients with history of previous IVF failure were studied. Oral estradiol valerate 4 mg/day was started on 28 patients on day 21 and continued till the 3<sup>rd</sup> day of menstrual cycle. Fifty-eight patients received estradiol valerate throughout the ovarian

stimulation period till the day of hCG administration. Both the luteal phase and stimulation phase estrogen treatment resulted in significant improvement in the ovarian responsiveness. This demonstrates that luteal phase estrogen treatment is beneficial in improving pregnancy rate in poor responders with previous failed cycles.

Tay and Lenton in his randomized study have shown the effect of estradiol on the luteal phase in addition to the progesterone.<sup>[18]</sup> Sixty-three women undergoing IVF with GnRH-antagonist (GnRH-a) and follicle stimulating hormone (FSH) and 55 women undergoing intrauterine insemination with clomiphene citrate and FSH were studied. The patients were subjected to progesterone (Cyclogest) with or without 2 mg of estradiol valerate during luteal phase. Pregnancy outcome results were similar in both the groups. Another study by Fatemi *et al.*, demonstrated the effect of estrogen therapy during luteal phase in stimulated cycles for IVF.<sup>[19]</sup> Ovarian stimulation was done on 201 patients with fixed dose of 200 IU FSH and GnRH-a. Patients were randomized and received 600 mg of micronized progesterone with or without 4 mg of estradiol valerate. Results showed that addition of estradiol valerate does not alter the pregnancy outcomes.

## G-CSF SUPPLEMENTATION AND IVF TREATMENT

G-CSF is a cytokine/growth factor crucial for implantation<sup>[20]</sup> and it is also a remedy for implantation failure.<sup>[21]</sup> In this review, all the studies regarding the G-CSF and IVF treatment were taken into consideration. We evaluated the success rate of IVF with supplementation of G-CSF in embryo culture media and also the success of women undergoing IVF with thin endometrium which fails to proliferate with standard treatment, but respond with transvaginal instillation of G-CSF into the endometrium. According to a randomized multicentric controlled double blinded study<sup>[22]</sup> of 1,332 women with IVF or intracytoplasmic sperm injection (ICSI); 1,149 had ET. After the fertilization of oocytes, the embryo was cultured and transferred to test media containing 2 ng/ml of G-CSF and control media. The outcome measured as ongoing implantation rate at 7<sup>th</sup> week with follow-up at 12 weeks and birth. The results were remarkable as the survival rate of the embryo cultured with G-CSF till 12<sup>th</sup> week and birth were significantly higher which proves the efficacy of the G-CSF in assisted reproduction technology. Lédée *et al.*, presented proof regarding the importance of quantification of follicular fluid G-CSF in ET decision.<sup>[23]</sup> It reflected the necessity of analysis and monitoring of follicular fluid G-CSF in embryo selection process because if used to an advantage, it will drastically reduce the cost of treatment in achieving a successful pregnancy.

G-CSF has also been employed in the proliferation of the thin endometrium which fails to respond with standard therapy. In a prospective study done by Gleicher *et al.*,<sup>[24]</sup> G-CSF was perfuse transvaginally into the uterus of four patients undergoing IVF with thin endometrium after standard endometrial preparation. This resulted in 7 mm of additional endometrial proliferation which was previously resistant to estrogen and vasodilators therapy and all the patients successfully underwent ET and conceived. This demonstrates G-CSF as an innovative remedy in patients with unresponsive, inadequate, and thin endometrium.

### MODULATION OF ABNORMAL NK CELLS ACTIVITY BY INTRALIPIDS AND IMMUNOGLOBULINS (IGS)

Immune disorders play a significant role in patients with recurrent pregnancy losses and elevated NK cell activity is one of the foremost etiologies associated with this condition. Coulam and Acacio observed an enhancement in live births with immunotherapy treatment in women exhibiting certain immunological risk factors.<sup>[25]</sup> In patients with recurrent pregnancy losses associated with elevated NK cell activity, several modalities of immunotherapy have been tried. De Carolis *et al.*, demonstrated an increased implantation rate with the administration of intravenous Igs<sup>[26]</sup> and Roussev *et al.*, reported from his study an increment in the implantation rate of patients with reproductive failure.<sup>[27]</sup> Many studies performed on patients with recurrent implantation failure after IVF-ET treatment has been abridged by Clark *et al.*<sup>[28]</sup> In his review of literature, he has shown that out of 10 controlled trials four showed improvement leading to enhancement of live births, whereas six did not show any improvement. In five trials, intravenous Igs were administered preconceptionally among which four showed major enhancement to the live births; whereas in five trials in which intravenous Igs were given after the pregnancy was established, no positive response was seen with the treatment.

In a number of studies it has also been seen that intravenous administration of intralipids also improves the rate of live births. Several studies have proven suppression of NK cell cytotoxicity by usage of intralipids in *in vivo* as well as *in vitro*. Roussev *et al.*, has shown suppression of cytotoxic NK cell functional activity by periodic intravenous intralipids administration on 50 patients. The results revealed suppression of NK cell activity in 39 patients (78%) during the 1<sup>st</sup> week of infusion and 11 (12%) showed suppression which was above the threshold level. They received second infusion dose after 2-3 weeks which showed the normalization in NK cell functional activity during the 1<sup>st</sup> week in 10 patients. Four patients received three doses of intralipid infusion in between in 2 weeks

after which the NK cell functional activity normalized. The duration of suppression of NK cell functional activity by intralipids also plays a major role in clinical practice. In 47 patients, the suppression effect lasted for a total duration of 6-9 weeks, in two it lasted for 5 weeks and in one patient it lasted for 4 weeks.<sup>[29]</sup> Therefore, from all these studies it can be concluded that both intralipids and Igs act as immunomodulators and can be used clinically in modulating the NK cell activity; although when the pregnancy outcomes treated with Igs and intralipids were compared the results was approximately similar.

### POTENTIAL NON-ANTICOAGULANT EFFECTS OF HEPARIN IN IVF

Heparin not only acts as an anticoagulant but also have immunomodulatory and anti-inflammatory effect. Heparin promotes the heparin binding epidermal growth factor (HB-EGF) and improves the outcome in post ET therapy. Di Simone *et al.*, illustrated the effects of low molecular weight heparin (LMWH) on the modulation of HB-EGF expression. The evaluation by the usage of tinzaparin and enoxaparin with the help of ELISA and Western blot test on HB-EGF secretion was performed. Results showed increment in the secretion of the HB-EGF by LMWH. It also promoted the survival rate of decidual cells by decreasing decidual cell apoptosis.<sup>[30]</sup>

Fluhr *et al.*, also demonstrated the effect of LWMH on decidual cells.<sup>[31]</sup> Endometrial stromal cells from hysterectomy samples were isolated. Progesterone and 17-beta estradiol was used for the decidualization of the cells and unfractionated heparin along with three different LMWH (enoxaparin, dalteparin, and certoparin) was added in the medium. Amplification of the insulin-like growth factor was noticed in the endometrial stromal cells using unfractionated heparin and all of three different LMWH.

D'Ippolito *et al.*, also studied the invasiveness, HB-EFG, and cysteine-rich angiogenic inducer 61 secretion by extravillous trophoblast cells (ETVC). The ETVC were extracted from the women with first trimester recurrent pregnancy losses. LMWH (tinzaparin and enoxaparin) were used to study the invasiveness *in vitro* by matrigel invasion assay. Results showed significant increase in HB-EFG and cysteine-rich angiogenic inducer 61 secretion primarily with tinzaparin usage. Results also reflected increment in the ETVC invasiveness.<sup>[32]</sup>

An observational retrospective study on 265 patients with history of at least two IVF/intracytoplasmic sperm injection cycles with implantation failure was done.<sup>[33]</sup> Out of them, 149 (56%) were primary infertile, 116 (44%) were secondary infertile; and their mean age was 36.3. They

underwent assisted reproductive cycles. The pregnancy rate in patients treated with LMWH was 29.52%, whereas in untreated patients the pregnancy rate was 17.19%. This study showed the beneficial effect of LMWH on the pregnancy rate. Contradictory on the other side a study by Berker *et al.*, showed that LMWH does not improve the pregnancy outcome significantly in patients with two or more implantation failure.<sup>[34]</sup>

## CONCLUSION

Recurrent pregnancy loss and recurrent implantation failure are mostly associated with immunological factors mainly due to abnormal NK cell activity. Role of intralipids and Igs therapy for modulating NK cell activity needs more evaluation and research. However, supportive role of progestogens and estrogens are also considered for better outcome.

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