

Promising Implantation Rate When Granulocyte-Macrophage Colony-Stimulating Factor Used for Recurrent Pregnancy Loss Patients

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ABSTRACT

Objective: To investigate fertilization, blastocyst formation, and implantation rates in recurrent pregnancy loss patients when Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) enriched media is used for embryo culture and Frozen Embryo Transfer (FET).

Methods: Embryos from 48 IVF cycles were cultured in either G-TL or Sage 1 Step GM-CSF with HSA prior to vitrification on day 5 or 6. On the day of FET, all embryos were thawed and placed in Sage 1 Step GM-CSF with HSA for 2 hours prior to transfer. Implantation was determined two weeks after each FET through serum hCG tests, with levels > 5mIU/mL considered successful implantation.

Results: The implantation rate of embryos cultured in G-TL and transferred in GM-CSF enriched media was 51.85%. When embryos were placed in GM-CSF enriched media for embryo culture and transfer, fertilization rate, blastocyst formation, and implantation rates were 80.2%, 46.6%, and 71% respectively.

Conclusion: The results of this study suggest that the use of GM-CSF media for embryo culture and transfer lead to promising implantation rates.

Keywords

Pregnancy loss, Blastocyst, Fertilization, Heparin treatment.

Introduction

Recurrent pregnancy loss (RPL), or the loss of two or more clinical pregnancies, is an emotionally taxing condition that affects 2% of couples [1]. RPL has several accepted causes, including genetic, immune, and uterine abnormalities; however, in 50% of cases, the cause is unknown. As current treatment protocols rely on diagnostic testing and treatment of known causes (e.g. pre-implantation genetic screening to ensure euploid embryo transfer and surgical correction of uterine abnormalities), the most effective way to treat unknown cases of RPL is unclear. Heparin treatment and immunotherapy have been suggested as potential treatments in these cases; however, as they have not been found to improve live birth rate and can have significant side-effects, they are not recommended for use in cases of unexplained RPL [2,3].

Recent studies have explored potential immune-related explanations for recurrent miscarriage. During pregnancy, the fetus acts as a semi-allogenic graft, with genetic contributions from both parents [4]. As such, the fetus contains paternal antigens foreign to the maternal immune system, and successful pregnancy requires the suppression of the maternal immune response. Despite this, the immune system must also retain an adequate level of strength to protect against infection. This control of the immune system likely stems from various mechanisms. For example, during pregnancy, the immune system shifts from the T helper type 1 (TH1) inflammatory immune response to the T helper type 2 (TH2) immune response, the latter of which is a more targeted system that better supports fetal development [5]. In women with recurrent pregnancy loss, several studies have observed that the TH1 to TH2 cytokine ratio remains high during pregnancy, with significantly higher blood levels of TH1 cytokines IFN- γ , TNF- α , and TNF- β [6]. These results suggest that cytokine levels may play

important roles in the success of pregnancies.

GM-CSF is a cytokine that is thought to support pregnancy by facilitating trophoblast cell growth, shifting the maternal immune system to a state that supports fetal growth, and promoting the production of leukocytes for the various tissue remodelling events that occur during pregnancy [7].

GM-CSF is produced by a variety of cells such as fibroblasts, endothelial cells, and T-cells, particularly during inflammatory or autoimmune responses [8]. The cytokine binds to receptors on hematopoietic cells to stimulate their growth and differentiation through various signalling pathways [9]. In the female reproductive tract, GM-CSF is produced under estrogen regulation, where it facilitates intercellular communication between leukocyte cells in the uterus and trophoblast cells of developing embryos to promote development and proliferation [10].

Support for the importance of GM-CSF in maintaining pregnancy also comes from studies that have found lower blood concentrations of the cytokine in patients with recurrent pregnancy loss [11], as well as others that have found a beneficial effect of subcutaneous GM-CSF treatment on pregnancy rates in patients with RPL [12]. These results encouraged us to explore whether exposing embryos to GM-CSF in vitro would have any beneficial effect on embryo development and/or implantation rates in patients with recurrent pregnancy loss.

Materials and Methods

This study was composed of two experiments which took place at our clinic. The first included RPL patients between 26 and 43 years that underwent frozen embryo transfer (FET) at our clinic between March and May 2018, with GM-CSF supplemented media used only during embryo transfer (n=21). The second experiment included cycles with RPL patients aged 27 to 43, which took place between January and June 2019 with GM-CSF media used for embryo culture as well as embryo transfer (n=27).

In-Vitro Fertilization

Controlled ovarian stimulation was achieved using an antagonist/agonist protocol, with doses tailored to patients. 36 hours following hCG administration, oocytes were retrieved under ultrasound guidance. Retrieved oocytes were incubated for two hours prior to cumulus-corona denudation and morphological assessment. Mature oocytes were then inseminated by Intracytoplasmic Sperm Injection (ICSI) and cultured in groups. Approximately 18 hours later, fertilization of inseminated oocytes was confirmed by the presence of two pro-nuclei, and fertilized oocytes were cultured in either G-TL media (VitroLife) equilibrated at 37°C at 5% O₂ and 5-6% CO₂ (pH 7.28) for 16-18 hours prior to use (experiment 1) or in Sage 1 Step GM-CSF with HSA (Cooper Surgical) equilibrated at 37°C at 5% O₂ and 5-6% CO₂ (pH 7.28) for 16-18 hours prior to use (experiment 2). Embryos were cultured uninterrupted until day 5 or 6, and then good quality blastocysts were vitrified for future FET in both experiments 1 and 2.

Frozen Embryo Transfer

On the day of FET, embryos were thawed and placed in Sage 1 Step GM-CSF with HSA equilibrated at 37°C at 5% O₂ and 5-6% CO₂; pH 7.28 for 16-18 hours prior to use. In both experiment 1 and 2, all embryos were transferred in GM-CSF supplemented media under ultrasound guidance.

Two weeks post-FET, serum hCG tests were administered with levels >5 mIU/mL considered successful implantation.

Results

The first experiment included cycles with embryos cultured in G-TL media and transferred in GM-CSF enriched media. All patients enrolled in this experiment returned back to the clinic for frozen embryo transfer. In this group, the implantation rate was 51.85%.

In the second experiment, embryos were both cultured and transferred in GM-CSF enriched media. Only 7 of 21 patients in this experiment have undergone elective single embryo transfer to date, the remaining 14 patients have not yet returned back to the clinic for transfer. Of the 7 patients that underwent FET, the implantation rate was 71%. Fertilization and blastocyst formation rates in this group were 80.2% and 46.6%, respectively.

Discussion

During pregnancy, cytokines play important roles in supporting fetal development and mediating maternal-fetal communication. GM-CSF is a cytokine present in the female reproductive tract that has been implicated in leukocyte recruitment for uterine tissue remodelling, modulating the maternal immune system to prevent rejection of the fetus, and supporting fetal development [13]. Recent studies have found lower blood-levels of this cytokine in patients with RPL, suggesting that deficiencies in GM-CSF may be detrimental for pregnancy outcomes [11].

The results of this study show that the addition of GM-CSF to transfer media results in promising implantation rates, and suggest that the use of GM-CSF enriched media during both embryo culture and transfer improves implantation rate relative to its use solely during embryo transfer.

These results are in accordance with the existing literature on GM-CSF and IVF outcomes, where culturing embryos in GM-CSF enriched media was found to increase fertilization rate [14], blastocyst development rate [15], blastocyst quality [15], clinical pregnancy rate [16], and reduced biochemical pregnancy rate [17] in patients with recurrent pregnancy loss. In mice, the addition of GM-CSF to culture media was associated with increased number of blastocysts, higher implantation rates, reduced apoptosis, and increased glucose uptake [7]. Mice studies have also found that the use of GM-CSF enriched media during culture also prevents the accelerated growth and increased obesity rates common to mice born from embryos cultured in vitro [18].

Our preliminary study provides encouraging results on the use of

GM-CSF in embryo culture. Future studies that investigate live birth rates and postnatal development are recommended.

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