Original Article

Treatment of refractory thin endometrium with autologous blood cell derivative (ABCD-Endosera): Advancing toward a next-generation of platelet-derived growth factors in frozen embryo transfer cycles: A pilot study

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Abstract

Study question: Can platelet derived growth factors be useful for refractory endometrium management Aims and Objectives: Tissue engineering traditionally stimulates cells using a single protein. For example use of G-CSF for endometrial regeneration. In contrast, natural tissue regeneration relies on cocktail of signalling molecules and growth factors. This study is to evaluate the regenerative effect of platelet derived growth factors (ABCD-Endosera) on refractory thin endometrium. **Material and Methods:** This was a retrospective self-controlled pilot study. Forty-one women who had two or more failed IVF cycles and refractory thin endometrium were enrolled in this study. The main inclusion criteria were endometrial thickness (EMT) of <7 mm after more than 2 cycles of conventional therapy. The subjects were treated with intrauterine infusion of autologous platelet-derived growth factors concentrate 3 times from menstrual cycle day 6 of their frozen-thawed embryo transfer (FET) cycle in addition to standard HRT protocols, and embryo transfer was performed 3 days after the final PDGF infusion. On the day of embryo transfer (ET) the endometrial thickness was found to be >7mm with a tri-laminar pattern in all the patients and subsequently ET was performed. Clinical pregnancy was determined by positive serum β-HCG, two weeks after ET and the presence of a fetal heartbeat in trans-vaginal ultrasound four weeks after ET. **Results:** Endometrial thickness showed significant improvement on the day of embryo transfer with a uniform triple-layer pattern. Clinical pregnancy was achieved in 17 out of the 41 women participating in the study, representing a 41.5% success rate, indicative of a potential enhancement in clinical pregnancy rates attributable to ABCD-Endosera treatment. The remaining 24 participants, constituting 58.5% of the sample, did not achieve clinical pregnancy. There were no reported instances of miscarriages, and each participant experienced a full-term delivery, underscoring the effectiveness of the treatment. Furthermore, while the sample size is small, still this study would become valuable and encourage proof of concept data to increase the research about this type of treatment. **Summary & Conclusion:** In conclusion, our pilot study offers initial evidence affirming the potential of ABCD-Endosera in addressing refractory thin endometrium. Utilizing this autologous concoction of platelet-derived growth factors has shown significant enhancement in endometrial thickness and receptivity, leading to encouraging pregnancy outcomes. Nevertheless, to reinforce these preliminary results and establish the optimum therapeutic protocol for ABCD-Endosera, comprehensive large-scale and rigorous studies are indispensable. The prospective benefits of ABCD-Endosera in ameliorating endometrial receptivity are crucial, especially considering the time-sensitive context of IVF procedures. This novel therapeutic strategy holds the promise of transforming the management of refractory thin endometrium, thereby increasing the probability of positive pregnancy outcomes for women facing infertility challenges.

Keywords: Refractory thin endometrium, Autologous Blood Cell Derivative, Growth factor concentrate, Frozen embryo Transfer, In vitro fertilisation (IVF)

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INTRODUCTION

The clinical landscape of infertility management has witnessed remarkable advancements, with *in vitro* fertilization (IVF) being one of the most significant. However, one persistent obstacle for successful IVF outcomes is refractory thin endometrium, an unresponsive endometrium that resists thickening despite conventional hormonal treatments, hindering successful embryo implantation.[1] This condition is identified by an endometrial thickness (EMT) of <7 mm, and it significantly undermines the pregnancy success rate in IVF cycles.[2-4] The pathological underpinnings of thin endometrium are multifaceted, encompassing ischemia, fibrosis, and inflammation, and they have been extensively reviewed in the past.[5] Despite our increasing understanding of these pathologies, treatment options for refractory thin endometrium remain suboptimal. Protocols involving extended estrogen administration, low-dose aspirin, and sildenafil citrate, while promising, often fail to yield consistent outcomes.[6-8] In light of this clinical challenge, regenerative medicine has surfaced as a potential approach for endometrial regeneration. Single growth factor (GF) therapies such as granulocyte colony-stimulating factor (G-CSF) have been explored for endometrial regeneration.[9] However, these singular therapies may oversimplify the complexity of tissue regeneration, potentially limiting their efficacy.[10] Stem cell therapies have demonstrated promising results in improving EMT and vascularization.[11] However, these interventions present their own set of challenges, including high costs, ethical concerns, and long-term safety apprehensions.[12,13] Platelet-rich plasma (PRP), an autologous concentration of platelets, has gained attention for its potential to improve EMT and receptivity.[14] The efficacy of both stem cells and PRP is attributed to the release of GFs and signaling molecules such as platelet-derived growth factors (PDGFs), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF) that are intrinsic to tissue regeneration and wound healing.[15-17] Nevertheless, PRP also contains proinflammatory components, which can potentially impact its efficacy negatively and compromise embryo implantation.[18,19] The inflammatory milieu within the uterine environment plays a pivotal role in the process of implantation. Excessive or chronic inflammation can impair endometrial receptivity and negatively impact implantation rates.[20] Hence, an improved version of PRP devoid of proinflammatory components can potentially enhance endometrial receptivity.[21]

Building upon this premise, we hypothesize that a refined autologous preparation of GFs derived from platelets [autologous blood cell derivative (ABCD-Endosera)], purged of proinflammatory components could offer a more efficacious approach to manage refractory thin endometrium. The therapeutic efficacy of such GF combinations has been demonstrated in various fields, including orthopedics, wound healing, and dermatology, with their ability to promote tissue regeneration and healing.[22-25]

MATERIALS AND METHODS

This pilot study was conceptualized with the objective to evaluate the regenerative effect of ABCD-Endosera on refractory thin endometrium in patients undergoing frozen embryo transfer (FET). The primary outcome of this study was to evaluate the effect of intrauterine infusions of ABCD-Endosera on EMT in patients presenting with refractory thin endometrium. The secondary outcomes encompassed assessing implantation rates, clinical pregnancy rates, live birth rates, and the reporting of adverse effects.

This was a multicentric study conducted at Indira IVF and Fertility Center, Udaipur, Bangalore, and Chandigarh, India. We conducted a retrospective analysis of data from 41 women, ranging in age from 22 to 47 years, for whom one or more embryo transfer cycle was cancelled due to thin endometrium (<6 mm) after optimum hormone replacement therapy [HRT (estrogen)] therapy before FET. This pilot study was

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executed over a span of 17 months from August 2018 to December 2019.

Inclusion Criteria

Patients must have a history of thin endometrium failed IVF due to inadequate EMT, often diagnosed through ultrasound imaging during ART cycles. A commonly used threshold is an EMT of <7 mm after optimum HRT.

(1) Poor responders to hormonal treatment: Patients who have not shown adequate endometrial thickening in response to standard estrogen therapy were included. This is usually characterized by EMT <6 mm despite extended estrogen administration prior to FET.
(2) Inadequate response to vasodilator therapy: Patients who have received treatments intended to increase blood flow to the endometrium, such as low-dose aspirin, sildenafil, or vitamin E, but still have thin endometrium.
(3) Failure of treatment with other growth factors or cytokines: Patients who have received therapies such as G-CSF that aim to improve EMT and receptivity but have not seen significant improvements were included.
(4) Failed response to hysteroscopic adhesiolysis: In some cases, thin endometrium is caused by intrauterine adhesions. Patients who have undergone hysteroscopic adhesiolysis (a surgical procedure to remove these adhesions) but have not achieved sufficient endometrial thickening were included.

The patients included in this study were treated at multiple locations with conventional treatment modalities, including hormone and vitamin therapy as well as treatment with drugs that improve blood circulation and conventional PRP where several cycles have been canceled due to insufficient EMT and did not have any significant impact on pregnancy rates.

Autologous Blood Cell Derivative-Endosera Preparation

ABCD-Endosera was prepared from autologous blood, as previously reported.[26] ABCD GF concentrate was prepared from concentrated platelets, prepared from fresh peripheral blood collected from a peripheral vein, stored in BD Vacutainer Acid Citric Dextrose (ACD) Solution A Blood Collection Tube, meant for clinical use, and processed to separate various blood components. Whole blood was then subjected to proprietary centrifugation-based selective enrichment protocol, and the upper fraction was separated, without disturbing the red blood cell (RBC) layer, and transferred into a sterile tube. A 100 µL sample was separated for determining platelet concentration and purity. The collected upper fraction underwent centrifugation for 12 minutes, after which the supernatant, referred to as platelet-poor plasma (PPP), was transferred into a sterile tube. The platelet pellet that was obtained was resuspended in PPP. The platelet fraction alone was concentrated further from the above separations and platelets were stimulated to release cytokines and GFs. Enriched GF concentrate was recovered by centrifugation at 3000 × g up to 20 minutes at 18°C, then filtered into three 1 mL doses with PPP. The platelet count measured by the hematology analyzer confirmed a nine-fold increase from the baseline in the concentration of platelets used to prepare the GF concentrate.

After obtaining informed consent, 0.8 mL of ABCD was administered on day 5, 6, or 7 of the menstrual cycle, or upon the complete cessation of bleeding on any of these days. As presented in Figure 1, three doses of ABCD-Endosera (0.8 mL per dose) were prepared from 30 mL of peripheral blood. Dose 1 was instilled with an intrauterine insemination catheter, and doses 2 and 3 were frozen for

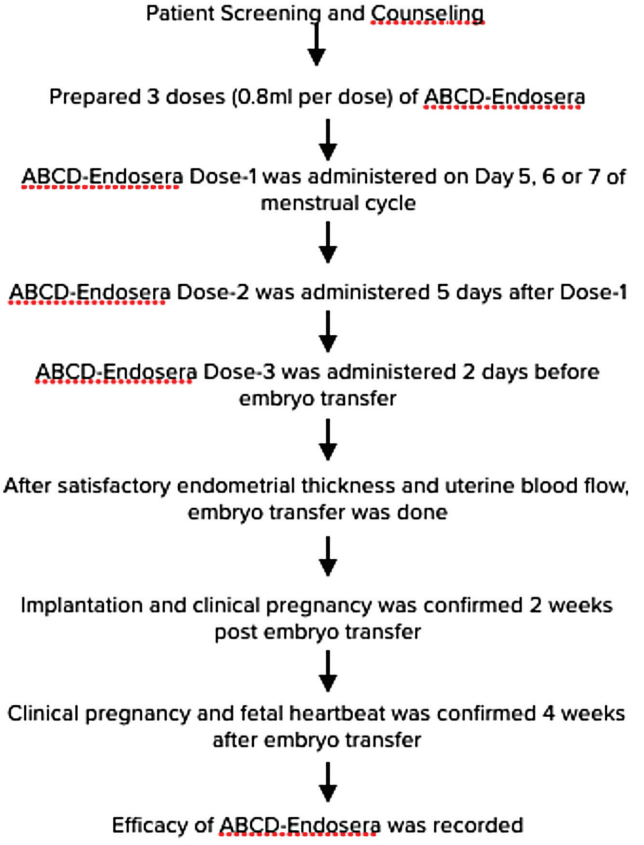

Figure 1: Preparation and administration of autologous blood cell derivative (ABCD)-Endosera.[26]

future use. The second dose was instilled 5 days after the first dose, and dose 3 was administered 2 days before embryo transfer. On the day of embryo transfer, EMT was observed to have increased to >7 mm with a trilaminar pattern in majority of patients. Endometrial and subendometrial blood flow, crucial for successful implantation, was monitored using color Doppler in 2D mode on a transvaginal scan. Prior to ABCD-Endosera instillation, these patients had no visible endometrial/subendometrial blood flow, whereas a noticeable improvement in blood flow was evident post-treatment as per the different zones of vascularity with reference to the Applebaum criteria.[27] Biochemical pregnancy was confirmed by positive serum beta-human chorionic gonadotropin (β-hCG) 2 weeks post-embryo transfer, and the clinical pregnancy was established by the presence of a fetal heartbeat as revealed by a transvaginal ultrasound 4 weeks post-embryo transfer.

Statistical Analysis

Data were collected using MS Excel and analyzed using SPSS statistical software (SPSS, Chicago, IL, USA) version 28.0. Categorical variables were expressed as numbers and percentages, while continuous variables were expressed as mean [standard deviation (SD)].

RESULTS

The demographic and baseline characteristics of the study participants are presented in Table 1. The mean age of the study participants was 36.07 years (SD ± 5.42) and mean body mass index (BMI) was 25.36 (SD ± 4.85). All women had a history of two or more cancelled IVF cycles due to persistently thin endometrium (<7 mm) despite conventional therapies. In this pilot study, intrauterine infusion of ABCD-Endosera was performed in 41 women with refractory thin endometrium. Significant improvements in EMT were observed. The mean EMT on the day of embryo transfer was significantly greater than the mean EMT of the previous cycles (mean difference = 1.6 mm, SD ± 0.4; $P < 0.001$). All the women exhibited a uniform triple layer endometrial pattern and change in Doppler flow parameters was not done. (Tables 2 and 3)

Clinical pregnancy was achieved in 17 out of the 41 women participating in the study, representing a 41.5% success rate, indicative of a potential enhancement in clinical pregnancy rates attributable to ABCD-Endosera treatment. The remaining 24 participants, constituting 58.5% of the sample, did not achieve clinical pregnancy. A noteworthy observation is that of the women who did achieve clinical pregnancy, there were no reported instances of miscarriages, and each participant experienced a full-term delivery, underscoring the effectiveness of the treatment. Furthermore, the absence of reported adverse effects throughout the duration of the study highlights the treatment's favorable safety profile. The results also indicated a β-hCG rate of 41.5%, an implantation rate of 30.1%, and notably, no biochemical losses or miscarriages, corroborating the potential of ABCD-Endosera in improving reproductive outcomes.

Table 1: Basic Demographical Analysis of Continuous Variables.

Name of Characteristics (n = 41)	Count	Mean	SD
Wife's age	41	36.07	5.42
BMI	41	25.36	4.85
AMH	33	3.64	6.16
Husband's age	41	38.24	6.02
Sperm count (M/mL)	35	49.34	25.99
Sperm motility (%)	36	44.69	23.40
Sperm morphology (%)	33	2.33	1.02
Oocytes retrieved	24	11.28	6.68
M-II	24	9.38	6.06
M-I	24	1.13	1.23
GV	24	0.88	1.26
Endometrial thickness at start of Gestone	41	7.09	0.54
Embryo age in days	41	5.07	0.26
No. of embryos transferred	41	1.78	0.42

AMH = Anti-Müllerian hormone, BMI = body mass index, GV = Germinal-Vesicle, SD = standard deviation.

DISCUSSION

PRP has emerged as an innovative treatment strategy in numerous medical fields, including orthopedics,[28] cardiothoracic surgery,[29] plastic surgery,[30] dermatology,[31] dentistry,[32] and diabetic wound

Table 2: Basic Demographical Analysis of Categorical Variables

Name of Characteristics (n = 41)	Categories	Count (n)	Percentage (%)
No. of embryos transferred	1	9	22.0
	2	32	78.0
Tran. grade	A	38	92.7
	B	1	2.4
	AB	2	4.9
B-hCG	Negative	24	58.5
	Positive	17	41.5

Table 3: *In Vitro* Fertilization Outcome

β-hCG rate	17/41	41.5%
Implantation rate	22/73	30.1%
Clinical pregnancy rate	17/41	41.5%
Biochemical loss rate	0/17	0.0%
Miscarriage rate	0/17	0.0%
Ongoing pregnancy rate	17/41	41.5%
Live birth rate	17/41	41.5%

healing.[33] Of late, PRP has started to gain attention in the realm of reproductive medicine, particularly in the management of infertility-related conditions.[34]

Several areas of reproductive medicine research have focused on the potential applications of PRP. These include its role in the treatment of poor ovarian reserve,[35] menopause,[36] premature ovarian failure,[37] and thin endometrium,[38] given its role in promoting tissue regeneration, angiogenesis, cell migration, differentiation, and proliferation.[39] This multifaceted role of PRP is attributed to the various GFs and cytokines it releases upon activation, including TGF-β, fibroblast growth factor (FGF), insulin-like growth factors 1 and 2 (IGF-1 and 2), VEGF, PDGF, and epidermal growth factor (EGF). VEGF and PDGF are particularly vital as they have been shown to stimulate angiogenesis, a process pivotal in both normal endometrial physiology and the repair of endometrial damage. Angiogenesis is essential in building a robust and receptive endometrium, thereby increasing the chances of successful implantation and pregnancy. TGF-β, meanwhile, is critical in regulating the formation of extracellular matrix, which is a fundamental component of tissue repair and regeneration.[40] It is suggested that high concentration of GFs and cytokines secreted from platelets can stimulate the mitogenesis and proliferation of endometrial cells or endometrial stem cells, and then activate endocrine–paracrine pathways for improving the endometrial response to promote embryo implantation and pregnancy.[41] This formed the basis for developing next-generation PDGF concentrate ABCD-Endosera. In this study, we analyzed the effect of intrauterine instillation of ABCD-Endosera in improving EMT for embryo transfer in patients with refractory thin endometrium.

Significant improvements in EMT were observed and all the women exhibited a uniform triple-layer endometrial pattern. Clinical pregnancy was achieved in 17 of the 41 women (41.5%). Importantly, among those who achieved clinical pregnancy, no miscarriages were reported, and all women delivered full-term babies. While the sample size is small, this study would become valuable and encouraging proof of concept data to increase the research about this type of treatment. For a physiologically highly regenerative tissues such as endometrium, a combination of GFs accelerating the natural proliferation of endometrial cells and enhancing the tissue remodeling (epithelial, endothelial, and stromal layer integrity) to get a good-quality trilaminar pattern is crucial. This is generally overcome by providing supraphysiological quantities of GFs. The positive outcomes of this pilot study mark an important stride toward a novel approach for managing refractory thin endometrium. Despite the limited sample size, our study provides encouraging preliminary evidence that lays the groundwork for further investigation into the therapeutic potential of ABCD-Endosera in this challenging clinical scenario.[42,43] The fundamental logic of using ABCD-Endosera, an autologous preparation of PDGFs, is anchored in the complex mechanism of physiological wound healing. During natural tissue repair, activated platelets aggregate at the injury site, releasing a cascade of bioactive molecules, including various GFs that are instrumental to efficient tissue healing and remodeling.[44,45] Single GF therapies have been employed in a wide range of clinical fields for tissue regeneration. However, this strategy may oversimplify the multifaceted, tightly regulated process of tissue repair, thereby potentially restricting its efficacy.[46,47] In the context of IVF procedures, where the timeline is of critical importance, the necessity for rapid and effective endometrial preparation is pivotal to avoid cycle cancellation.[48] ABCD-Endosera delivers a synergistic mix of GFs that may better replicate the natural healing environment than a single GF therapy. By applying a supraphysiological dose of GFs, it might stimulate endometrial cell proliferation and enhance tissue remodeling, leading to the development of a robust trilaminar endometrium, a key feature of endometrial receptivity.[49,50] Nevertheless, the available literature supporting the beneficial effects of multiple GFs in enhancing endometrial receptivity remains limited, emphasizing the need for more rigorous investigations. While the study of PDGFs in endometrial growth and receptivity has the potential to significantly advance our understanding of reproductive biology, several challenges, and limitations need to be addressed in future research.

One challenge lies in the inherent complexity and redundancy of GF signaling pathways, which may make it difficult to pinpoint the specific contributions of individual GFs to endometrial function. To overcome this issue, future studies may benefit from employing advanced molecular and systems biology approaches, such as transcriptomics, proteomics, and metabolomics, to comprehensively profile the endometrial microenvironment and identify key regulatory networks and signaling hubs. Another limitation in the field is the reliance on animal models, which may not fully recapitulate human endometrial physiology and

pathology. The development of more physiologically relevant *in vitro* models, such as three-dimensional endometrial organoids, may help to bridge this gap and provide a more accurate representation of human endometrial function.[51]

Lastly, the translation of basic research findings into clinical applications remains a significant challenge, as many GF-targeted therapies have shown limited efficacy or undesirable side effects in clinical trials.[52] Further optimization of these therapies, including the development of more specific and targeted drug delivery systems, may help to improve their safety and efficacy in the treatment of reproductive disorders.[53]

CONCLUSION

In conclusion, our pilot study offers initial evidence affirming the potential of ABCD-Endosera in addressing refractory thin endometrium. Utilizing this autologous concoction of PDGFs has shown significant enhancement in EMT and receptivity, leading to encouraging pregnancy outcomes. Nevertheless, to reinforce these preliminary results and establish the optimum therapeutic protocol for ABCD-Endosera, comprehensive large-scale and rigorous studies are indispensable. The prospective benefits of ABCD-Endosera in ameliorating endometrial receptivity are crucial, especially considering the time-sensitive context of IVF procedures. This novel therapeutic strategy holds the promise of transforming the management of refractory thin endometrium, thereby increasing the probability of positive pregnancy outcomes for women facing infertility challenges.

FUTURE DIRECTIONS

Building upon the promising results of this initial investigation, future research endeavors should focus on conducting randomized controlled trials with larger and more diverse cohorts to validate the efficacy and safety of ABCD-Endosera. Additionally, investigations into the optimal dosage, frequency, and duration of ABCD-Endosera administration will be essential to refine the therapeutic protocol. Comparative studies evaluating ABCD-Endosera against other established treatments for thin endometrium will provide insights into its relative benefits and potential role in clinical practice. Lastly, a closer examination of the underlying mechanisms by which ABCD-Endosera enhances endometrial receptivity will contribute to a deeper understanding of its therapeutic potential and inform the development of improved strategies for managing infertility related to endometrial conditions.

Ethical considerations

The study was accepted by Institutional Ethics Committee with approval number DRI/ IMS.SH/ SOA/ 2021/127.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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