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Successful Implantation and Live Birth Following Autologous Platelet-rich Plasma Treatment for a Patient with Recurrent Implantation Failure and Chronic Endometritis

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Abstract. *Background/Aim: Patients diagnosed with chronic endometritis (CE) may fail to respond to standard antibiotic treatment. The driver behind the approach reported here was the imperative need for alternative therapeutic solutions. Case Report: This case report presents a woman with CE and premature ovarian insufficiency having experienced repeated implantation failures following donated embryo transfers. The patient was diagnosed with CE through hysteroscopy, microbiological analysis and scanning electron microscopy. Following the suggested antibiotic treatment, she underwent a new embryo transfer, but with subsequent pregnancy loss. Following a second antibiotic scheme, all diagnostic procedures certified the persistence of CE. The patient underwent autologous, intrauterine platelet-rich plasma treatment and a subsequent embryo transfer. The diagnostic procedures indicated no signs of CE, while the embryo transfer resulted in a twin pregnancy and birth. Conclusion: Platelet-rich plasma may be employed as a first-line CE treatment, especially for patients who fail to respond to conventional antibiotic schemes.*

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Chronic endometritis (CE) is a persistent inflammatory condition of the inner lining of the uterine cavity. It is characterized by infiltration of plasma cells and lymphocytes present in the endometrial stroma, increased cell density of the stroma, asynchronous maturation of epithelial cells and stromal fibroblasts, as well as by superficial mucosal oedema. The inflammation is often asymptomatic or oligosymptomatic with abnormal uterine bleeding and vaginal discharge, lower abdominal pain, fever, leukorrhea and menstrual dysfunction (1). A growing body of evidence points to patients with endometriosis presenting more frequently with CE further associated with infertility of unknown aetiology, unexplained repeat pregnancy losses and repeat embryo implantation failures following *in vitro* fertilization (IVF) treatments (2-5). Endometrial receptivity in CE can be defined as dysfunctional, since the local expression of numerous genes encoding cytokines, chemokines, apoptotic proteins and adhesion molecules is dysregulated (6-8). On the subject of CE and implantation failure, decidualization and the mechanism of its modification may provide an explanation. The process of decidualization takes place in endometrial stromal cells. Decidua differentiates to maintain pregnancy, while it produces growth factors, hormones and cytokines (9). Its role may be pivotal in regulating trophoblast invasion and the challenge presented by the maternal immune system (10, 11). Therefore, the association between CE and implantation failure may be provided given the role of the decidua on implantation and the fact that CE appears to modify the process of decidualization (9).

CE is considered to be attributed to ascending infection caused by organisms of the indigenous vaginal flora, sexually-transmitted infections, and tuberculosis, as well as through medical procedures that enable a pathway for bacteria to enter the uterus through the cervix. In the

endometrium in cases of CE, the most commonly detected microorganisms are Streptococcus species, *Corynebacterium*, *Escherichia coli*, *Enterococcus faecalis*, *Mycoplasma genitalium*, *Bacteroides bivius* and *Ureaplasma urealyticum* (12, 13), while earlier studies by Haggerty *et al*. also supported the implication of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in CE onset (14). Finally, fungal agents have been reported rarely as being responsible for infectious causes of CE (15). Immune response due to the presence of microbial infections is described to be relatively atypical of the mucosal tissue environment, causing infiltration of circulating B-cells in the endometrial stromal and glandular epithelial areas. Endometrial B-cells are normally located within the basal layer, while their accumulation in the functional layer is caused by pro-inflammatory molecules that are abnormally expressed (8).

Oral antimicrobial regimes are considered to be the gold standard in the treatment of CE. Although antimicrobial agents eliminate endometrial stromal plasmacytes, the endometrial/intrauterine microbial profile alterations of patients with CE remain unclear. A wide spectrum of antibiotic treatments has been proposed for CE such as doxycycline, ciprofloxacin, metronidazole, ofloxacin, amoxicillin, clavulanate, josamycin and minocycline. However, certain CE cases do not respond satisfactorily to these widely applied treatments, driving research towards addressing the need for alternative options when managing these cases (2).

Platelet-rich plasma (PRP) use in the treatment of various medical cases, including osteoarthritis and tendonitis, has been introduced due to its antimicrobial and anti-inflammatory properties (16, 17). In light of the above findings, we sought to explore whether autologous, intrauterine PRP treatment might represent a treatment for CE. PRP is prepared by peripheral blood withdrawal following centrifugation in order to achieve a high concentration of platelets (18). Platelets carry more than 800 proteins, namely cytokines, hormones and chemoattractants of stem cells, macrophages and neutrophils, responsible for various post-translational modifications of nearly 1,500 bioactive factors (19). Platelets also carry numerous growth factors of great significance due to their ability to repair tissue as well as their mitogenic, chemotactic, neovascular and anti-inflammatory effects (20). These factors include: vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), keratinocyte growth factor (KGF), connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF), insulin-like growth factor 1 (IGF-I), epidermal growth factor (EGF) and 2 (IGF-II) and hepatocyte growth factor (HGF), which are released following alpha granule activation by native or exogenous molecules, including thrombin, collagen, magnesium and calcium chloride. The aforementioned proteins play fundamental roles regarding inflammation control, tissue regeneration, angiogenesis activation, anabolism increase as well as cell migration, differentiation and proliferation (21-23). Further to the above, supplementing cell cultures with PRP has been suggested by various research groups due to its ability to induce cell proliferation (24-26).

Case Report

In the current study, a 35-year-old woman, with premature ovarian insufficiency and a history of six failed donated embryo transfers, was referred to our clinic for assisted reproduction. The patient's detailed medical history was recorded and blood samples were taken. Molecular testing excluded thrombophilia. Thorough assessment of metabolism and hormonal function including glucose tolerance test, prolactin and thyroid function tests were also performed.

Hysteroscopy was performed during the luteal phase of the menstrual cycle of the patient, employing a lens-based 3 mm OD mini-telescope, 1058 angle of visual field equipped with a 3.5 mm OD single-flow diagnostic sheath. The required uterine cavity distention was performed with the use of saline, while a 300 W light source with a xenon bulb, a digital camera and a 21-inch video screen were employed. The cavity was panoramically evaluated assessing the endometrial mucosa. The presence of micropolyps, polypoid endometrium, stromal oedema and diffuse hyperaemia was taken into account and CE was the established diagnosis. A 3 mm Novak curette connected to a 20-ml syringe was employed in order to perform biopsy of the endometrium and analyse infectious agents. Contamination risk was controlled employing a vaginal speculum and by cleaning the external uterine ostium with iodine solution. Novak cannula application was performed under visual control, aiming to avoid contact with vaginal walls. Endometrial samples were placed into two 2-ml aliquots of saline for microbiological and scanning electron microscopy (SEM) analyses. Literature does not report on SEM analysis as a diagnostic tool with regards to CE. In the context of this work, SEM evaluation provided practitioners with supplementary data pertaining to the endometrium's overall condition. The microbiological analysis evidenced the presence of *Candida albicans*. The second aliquot was placed in neutral formalin and later embedded in paraffin for SEM examination. SEM revealed increased plasma cell concentrations and mycoplasma accumulations in the endometrial stroma of the patient, confirming the hysteroscopic and microbiological findings.

Based on the infectious agent detected, an appropriate antimycotic treatment was prescribed. Specifically, the patient received 800 mg fluconazole daily for 7 days according to the recommendations published by the Infectious Diseases Society of America (27). Following the antimycotic therapy and suitable hormonal treatment, the patient underwent

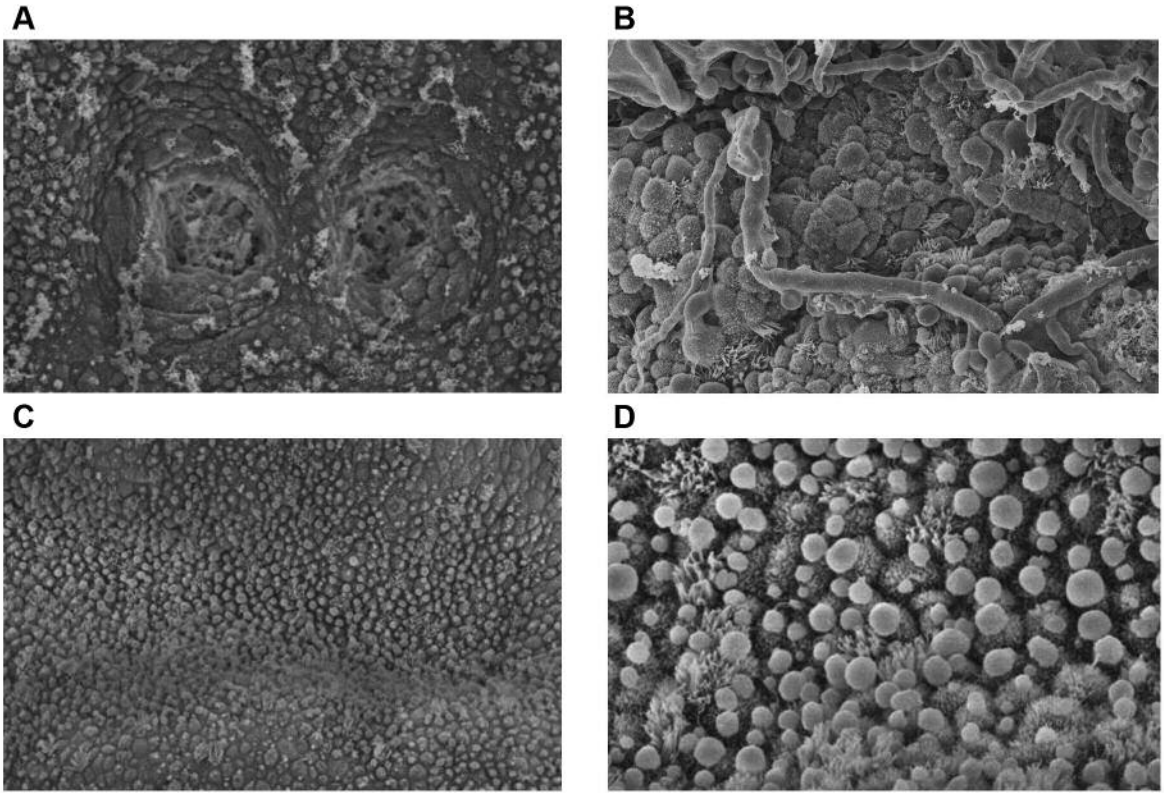

Figure 1. *Scanning electron microscopy analysis of the patient's inner endometrial lining at the luteal phase of the menstrual cycle after the second antimycotic scheme (A and B) and after platelet-rich plasma (PRP) treatment (C and D). A: Two gland orifices of the infected endometrial epithelium are visible, containing mucus and blood cells. Clusters of mucus are also present on the epithelium surface (magnification ×750). B: Fungal spores, red blood cells and mucus in endometrial biopsy. The hyphae are in contact with the epithelial cells, fusing with some of them (magnification ×2,700). C: Normal endometrial epithelium morphology was apparent following PRP treatment (magnification ×750). D: Presence of pinopodes in the cured endometrial epithelium following PRP treatment (magnification ×2,700).*

transfer of two donated blastocysts. Blastocyst grading was performed according to the criteria of Gardner *et al*. (28). The two blastocysts were graded as 5AA and pregnancy was confirmed 2 weeks following embryo transfer by human chorionic gonadotropin (β-hCG) quantification. Although β-hCG was increasing normally, abnormal bleeding and subsequent pregnancy loss took place 20 days post-embryo transfer. A second administration including the same antimycotic scheme as previously employed, was performed for the same period of time. Following the above treatment, as well as appropriate endometrial preparation, the patient underwent a second hysteroscopy at the luteal phase of the menstrual cycle for CE signs, while endometrial samples for SEM and culture were collected. All analyses confirmed the persistence of CE, while SEM revealed increased inflammation (Figure 1A and B).

Taking into account the effectiveness of PRP on different inflammation types, we performed an autologous, intrauterine PRP treatment. In accordance with the Helsinki declaration, the present study protocol was approved by the Hospital Ethics Committee (reference number: 11/8-1-2018). Following informed consent for the treatment from the couple, preparation of the autologous PRP was performed during the follicular phase of the cycle employing RegenACR®-C Kit (Regen Laboratory, Le Mont-sur-Lausanne, Switzerland) following instructions provided by the manufacturer. The volume of peripheral blood required to yield approximately 2.5 ml of prepared PRP for infusion for this patient was 25 ml. The initial density of platelets in the peripheral blood sample was 250,000/μl while that of the prepared PRP was 900,000/μl. Per our protocol, prepared PRP was allowed to be stored for 1 h at 4˚C. However, for this case it was administered immediately following preparation. The endometrial infusion was performed employing transvaginal ultrasound guidance and use of an appropriate catheter (Gynetics Medical Products N.V., Lommel, Belgium). The volume of PRP infused into the uterine cavity was approximately 2.5 ml.

Following PRP treatment and endometrial preparation, a third hysteroscopy was performed at the follicular stage of the subsequent menstrual cycle, while an endometrial sample

was extracted at the respective luteal phase. Subsequent hysteroscopy, microbiological analysis and SEM (Figure 1C and D) showed no signs of CE.

Our patient underwent another embryo transfer of two donated, blastocysts graded as 5BB and 5BC at the next menstrual cycle, which resulted in a twin pregnancy. Four weeks following a positive β-hCG pregnancy test, clinical pregnancy was confirmed by observing foetal cardiac activity on transvaginal ultrasound. The babies were delivered at the 36th week of gestation and weighed was 2.28 kg and 2.18 kg.

Discussion

This case report uniquely presents a patient with CE who failed to respond to the standard approach of antibiotic regime and who was successfully treated for CE following an autologous intrauterine PRP treatment. CE in this case was associated with infertility due to premature ovarian insufficiency and recurrent implantation failure with donated embryos. This case was effectively managed employing autologous intrauterine PRP, which in turn allowed successful treatment of CE and enabled subsequent implantation of transferred embryos leading to live birth of twins. Although the development of medically assisted reproduction technology has presented a valid solution to the issues of female infertility, a considerable proportion of infertile women still fail to be successfully managed with respect to achieving a pregnancy. Recurrent implantation failures and pregnancy losses have either maternal or foetal aetiology (29). Among the most frequent maternal causes are thrombophilia, abnormal uterine anatomy, impaired endometrial receptivity, as well as various immunological factors (30). Unfortunately, the above aggravating factors are usually diagnosed *a posteriori*. CE, which has direct effects on IVF success and pregnancy maintenance, is often asymptomatic and undetectable by ultrasound or hysterosalpingography.

Elimination of inflammation of the uterine stroma constitutes the basis of CE therapy. Various mechanisms may serve this aim, such as the augmented apoptosis of pro-inflammatory cells (31), the diminished expression of a considerable list of factors, including prostaglandins, pro-inflammatory cytokines (IL-1, TNFα) and matrix metalloproteinases, as well the heightened expression of anti-inflammatory molecules, including interleukin 1 receptor antagonist and tumor necrosis factor receptor (TNFR). To add to this list, the synthesis and release of anti-inflammatory cytokines (IL-4, IL-10) (32), TGF-β (33) and lipoxins (34) contribute to managing successfully uterine stroma inflammation along the therapeutic basis of CE. Furthermore, the cleavage of chemokines with matrix metalloproteinases has been found to trigger the production of anti-inflammatory factors, while the increased cell survival in the inflammation regions caused by interacting with the extracellular matrix frequently contributes to terminating the inflammation (35).

Intrauterine PRP treatment may activate many of the above processes. Applying platelet-derived factors into areas of damaged tissues could endorse healing renewal and regeneration (36-38). The bioactive nature of these factors consisting of coagulation factors, proteins with antibacterial and fungicidal effects and membrane glycoproteins directly and proportionally affects interleukins and chemokines synthesis, reported to enable management of inflammation symptoms (39). Further on, delineating the healing pathway triggered by PRP application through tissue homeostasis, stimulation is ensured by exposure to dense granule-derived factors, such as adenoside diphosphate, adenoside triphosphate, serotonin, histamine, dopamine and calcium ions as reported by Zhu *et al*. (39), whereas alpha granule-released growth factors are profoundly crucial in achieving wound healing and tissue regeneration (40). A recent study, examining mares with chronic degenerative endometritis, has shown that uterine PRP infusion as an immunomodulator of the inflammatory response, can modulate the local inflammatory response by reducing the number of polymorphonuclear neutrophils into the uterine lumen and preventing intrauterine fluid retention (20). In addition, the intrauterine PRP treatment has been showed to promote endometrial growth improving the pregnancy outcome of patients presenting with thin endometrium due to the growth factors PRP consists of such as vascular endothelial growth factor, platelet-derived growth factor, transforming growth factor beta, insulin-like growth factor, epidermal growth and fibroblast growth factor 2, factor that stimulate the healing process (41-43). *In vitro* results of a very interesting study on the potential of PRP to act as promoter of follicular development strengthen the hypothesis that PRP is a most dynamic source of growth factors effectively supporting viability, regeneration and growth (44). In light of the above, it may be extrapolated that autologous, intrauterine PRP treatment on this patient probably modulated the aberrant inflammatory processes of CE while promoting healing of the endometrial stroma.

The underlying mechanisms through which PRP acts as a modulator alleviating inflammation are further strengthened by *in vitro* studies of PRP treatment on animal models (20, 45). Proliferation as well as gene expression of endometrial cells have already been demonstrated and confirmed by *in vitro* bovine studies. However, the mechanism by which this gene regulation is achieved deserves to be further investigated. Although, the molecular mechanisms still belong in the grey zone, it is believed that the PRP-derived growth factors play the leading role. A study by Marini *et al*. demonstrated that the expression of *c-MYC* gene was up-regulated in endometrial cells which were cultured with PRP, in comparison to the expression levels found in untreated cells (45). *c-MYC* is involved in cell

proliferation and growth, and is activated by EGF, a component of PRP. This is one representative of many examples of the effects of PRP, demonstrating its effectiveness in reducing gene expression of pro-inflammatory factors such as interleukin 1β, interleukin 8, prostaglandin-endoperoxide synthase 2, and inducible nitric oxide synthetase, revealing its promising prospectives in *in vivo* regenerative therapy in endometritis (45). El-Sharkawy *et al*. reported that treatment with PRP increased the release of various growth factors such as insulin-like growth factor-I, fibroblast growth factor, epidermal growth factor and transforming factor β (46). The same study of the response of the injured area highlighted the presence of vascular endothelial growth factor as well as a local increase in lipid molecules (46). In addition, PRP also increased chemokine expression, the importance of which relates to the fact that these molecules can inhibit the relocation of leukocytes around the tissue affected (47). An *in vitro* study on mares by Reghini *et al*. reports similarly solid data of the ability of PRP to reduce the influx of polymorphonuclear neutrophils (20).

Published data indicate that PRP infusion may be employed for the distinct category of women presenting with poor ovarian reserve or response. The use of PRP in the reproductive context was pioneered by our team and cited by Sills and colleagues (48). Further to that, two case series have been published reporting on ovarian rejuvenation following intraovarian PRP infusion in women characterized as either peri-menopausal (49), or of poor ovarian response (50). Regarding this case report, what should be further highlighted as strengthening the cause and effect association between PRP and treatment of CE enabling pregnancy and live birth is the fact that the quality of the donated embryos that led to a miscarriage was in fact superior when compared to the quality of the embryos that actually resulted in live birth following PRP treatment.

This study uniquely documents that autologous intrauterine PRP treatment holds the potential of being employed as a successful therapeutic tool for CE. Verifying our findings in larger patient groups through randomized controlled studies would strengthen this finding and secure the role of PRP as a successful therapeutic means for patients with CE, especially for those that fail to respond to conventional antibiotic schemes. The current approach was successful on two intertwined levels as infertility was addressed and treated following PRP treatment of CE, allowing for the successful implantation of two donated embryos leading to subsequent clinical pregnancies and live births.

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