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Management of Resistant Endometrium in Cases of Recurrent Implantation Failure Using Endometrial Mesenchymal Stem Cells as an Innovative Regenerative Therapy-A Short Communication

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Abstract

Currently the most accepted definition of Recurrent Implantation Failure (RIF) is the absence of achieving clinical pregnancy following transfer of 3 or more good quality embryos in women under 35 years age as well as 4 or in ≥ 35 years age women in fresh or frozen ET's. We had reviewed earlier comprehensively how to manage the endometrial factor in cases of RIF utilizing antibiotics not only orally but further using intrauterine antibiotics and then Platelet Rich Plasma (PRP). Despite that there are certain cases that refuse to respond. We have further delved deeper into pathophysiology of Recurrent Implantation Failure (RIF) along with describing innovatively the use of Mesenchymal Stem Cells (MSCs) cells derived from endometrial stem cells in 29 cases of RIF mixed with PRP that was successful in 23/29 cases besides improving Endometrial Thickness (EMT), but further in Clinical Pregnancy (CP) as well as Live Birth Delivery Rates (LBDR). Further we describe the role of Platelet and Endothelial Cell Adhesion Molecule 1 (PECAM) along with Transforming Growth Factor Beta (TGF β) besides CDYL in RIF.

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Keywords

Recurrent Implantation Failure; Chronic Endometritis; Clinical Pregnancy; Live Birth Delivery Rates; Mesenchymal Stem Cells

Introduction

The optimal Endometrial Thickness (EMT) for conception remains controversial Endometrium Thickness (ETM) <7mm on Ultrasonography (USG) is usually thought to be suboptimal for Embryo Transfer (ET) [1]. About 0.6-0.8% of patients don't reach minimum EMT needed for embryo transfer (ET) [2]. Earlier we reviewed various causes of Recurrent Implantation Failure (RIF) along with Chronic Endometritis (CE) and how we can target via antibiotic therapy delivered in endometrium directly besides orally along with role of Platelet Rich Plasma (PRP) in cases of RIF [3-5]. Here we have tried to address what might be done if all the above fails. The possible reasons of thin Endometrium are:

- Inflammatory causes (acute/chronic endometritis)
- Iatrogenic (repeated curettage polypectomy)
- Hysteroscopic myomecomy or laparoscopic (in which cavity gets opened), as well as irrational use of Clomiphene Citrate (CC) [6]
- Further thin Endometrium might be due to individual uterine structure pattern [7].

Despite lots of therapy, most of these only get minor changes in EMT-hence they have not been validated [1]. Angiogenesis is the generation of new blood vessel via existing vascular structure through elongation, intussusception or sprouting of endothelial cells, as well as following birth vascularisation gets determined as well as sustained by angiogenesis [8]. Physiological angiogenesis does not take place in most organs in adult. But endometrium is the area, where normal angiogenesis occurs, being a fundamental event in normal menstruation as well as embryo implantation pointing that endometrial angiogenesis takes place by elongation, intussusception or instead of sprouting generation [9,10]. Blood vessels are made up of inner endothelial cell layer that lines the vessel wall as well as perivascular pericytes, also called mural cells that envelop the vascular tube surface.

Pericytes are multipotent cells which are heterogenous in their origin, function, morphology as well as surface markers [8]. Evaluation of the anatomic association among pericytes as well as endothelial cell demonstrate that they can crosstalk through juxtacrine or paracrine signal [11]. Pericytes were documented to respond to Platelet Derived Growth Factor B (PDGFB) as well as Transforming Growth Factor Beta (TGF- β) that get liberated via platelets following injury [12]. This chemotactic response to PDGFB results in migration of pericytes to the outer layer of blood vessel. This migration aids the endothelial cells to proliferate at the wound site in response to vascular VEGF that is a significant controller of this event [13]. Lots of studies

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have documented that VEGF gets expressed differentially in thin endometrium [14-16]. Uterine Natural Killer (uNK) cells are the main source of cytokines as well as angiogenic growth factor that includes VEGFA, Placental Growth Factor (PLGF) as well as angiopoietin that might form cytokines for facilitating angiogenesis at time of embryo implantation [17,18]. Despite uNK cell counts being enhanced in women with Recurrent Miscarriages (RM) as well as RIF, angiogenesis seems to be paradoxical in the 2 groups of ladies [19]. Earlier publications identified CD56+ uNK cell from women with RIF develop minimum amounts of angiogenic factors, like VEGFA, PLGF, PDGFB as compared to women with Recurrent Miscarriages (RM) as well as fertile controls [20]. It has been posited that angiogenesis might be decreased by stimulating the STATs pathway in uNK cells in women with RIF [21]. Enough proof of the presence of stem cells SC's in the human endometrium as well as the chances that they may be a treatment source in endometrium atrophy, thinned endometrium as well as Asherman syndrome [22]. The ability to sustain a normal karyotype following various passages, differentiate into various cell lines under standard culture its immunosuppressive capabilities inhibits LT, LB as well as NK [23-26]. Endometrial Mesenchymal Cells (enMSC's) a resource of significance in some regenerative therapies. These immunomodulatory characteristics get answered via the liberation of inflammatory cytokines in the tissue [27].

Besides that the low immunogenic ability as well as tumor generating capacity makes it the best option clinically [28]. One's significant property is that both infection as well as inflammation might prevent regeneration of damaged endometrium via damage to the stem/progenitor cells through effector molecules that also participate in the deposition of fibrotic tissue [29]. Oocyte donation cycles are the best to measure independent influence of endometrium receptivity, since variability in embryo quality is less RIF represents a clinical condition that points to a condition when implantation has failed repeatedly to arrive at a point when one can recognize via Transvaginal Sonography (TVS). Hence is a very difficult situation for the patient as well as treating doctor.

For trying to pinpoint the endometrium as the major etiological factor for RIF, various embryonic condition as well as number of consecutive cycles have been posited. Usually impossibility of getting a clinical pregnancy following 3 consecutive *In-vitro* Fertilization (IVF) procedures, where 1 or 2 embryos of good quality embryos got transferred in every cycle [30]. The exact definition continues to be controversial, hence other ones have been suggested [31]. The most accepted one currently is the absence of achieving clinical pregnancy transfer of 3 or better quality embryos in women under 35 years age as well as 4 or in ≥ 35 years. age women in fresh or frozen ET's [32]. In cases of CE the immune responses are usually switched towards proinflammatory profiles and thus not favourable for invading embryos [33]. The greater correlation among CE along with RIF has been proved (14-31%), of unknown reasons (28%) as well as RPL (9-13%) [34-37]. Recent published articles demonstrated that 34.4% of women with RIF as well as CE, that is > of women with RPL [38]. The influence of CE on perinatal results has been taken into account, taking Clinical Pregnancy (CP) as well as Live

Birth Rates (LBR) with/without treatment (56 vs 7%) into consideration [39]. It has been established that CE gave markedly low TGF- β as well as IL-10 expression in the endometrium that points. Treg cells in a number of functional deficit [38]. CE Control is so complicated that the bacteriological clearance is inefficient as a criteria of cure that might answer the high incidence of persistence CE observed (24.6% as well as 17.6%) [40,41].

Hence, Tersoglio et al., examined the endometrial alterations prior to as well as following transfer of enMSC's in a population of thinned endometrium women with lack or hyporesponsiveness to estrogen as well as RIF. Secondary objective was to analyze the clinical outcomes for the intervention with regards to Clinical Pregnancy Rate (CPR) as well as Live Birth Delivery Rate (LBDR) per *In-vitro* Fertilization (IVF) cycle. A longitudinal as well as experimental study the intervention definitely was subendometrial inoculation of enMSC' as well as post intervention alterations were examined by these variables, EMT, Endometrial Flow Cytometry (EFC), Endometrial Histopathology (EHP) as well as Endometrial Immunohistochemistry (EIH). The variables were evaluated following the intervention (post treatment) as compared to earlier values pre-treatment. EMT values prior to and following treatment with enMSC' were 5.24 ± 1.24 mm vs 9.93 ± 0.77 ($p=0.000$) respectively. EFC demonstrated variations favouring normalized variables in the post treatment evaluation correlated with the pre-treatment ,LT/Li, LB/Li, NK/Li, CD8/CD3+ as well as CD4/CD8 had NS ($P=0.167$ as well as 0.118). A similar evaluation was done on EHP with an HP enhanced post treatment ($p=0.007$). The CP rate was 79.31% (23/29), a LBDR/ET was 45.45% (10/22) as well as ongoing pregnancy 7/29 (24.14%). Thus concluding that sub endometrial enMSC's inoculation facilitates a significant enhancement in EMT, normalize of the EHP, EIH. Hence IVF following treatment with enMSC's gives a very good rate of CP as well as LPDR (Fig. 1 and 2) [42].

Figure 1: Staining of tissue samples showing (A) Plasmatic Cells, (B) Stromal edema and elevated Stromal density, (C) Spindle Stromal cells, (D) Micropolyps, (E) Normal CD138, (F) High CD138, (G) High CD56 and (H) High CD20.

Figure 2: Individual evolution of endometrial thickness.

Further Esmailzadeh et al., tried with the aim that that endometrial mesenchymal stem cells (eMSC) have a vital role in regeneration of endometrium during menstrual cycles. Since it has been suggested that (eMSC) likely play a role in uterine receptivity and establishment of pregnancy, they attempted to evaluate the expression levels of five most known receptivity markers-Integrin (ITG) $\beta 1$, Rac1, HoxA11, ITG $\beta 3$ and Noggin-in eMSC of Recurrent Implantation Failure (RIF) and non-RIF women. They isolated human eMSC from Menstrual Blood (MB) of RIF and non-RIF women. The isolated eMSC characterized based on their morphological and behavioral characteristics, expression of MSC-specific surface CD markers and their capacity of differentiation into osteocytes and adipocytes. The expression levels of the five mentioned receptivity markers were analyzed with real time reverse transcription polymerase chain reaction. They observed that RIF and non-RIF eMSC expressed all tested genes at different levels. ITGb1 expression in RIF, eMSC was lower than its expression in non-RIF cells. On the other hand, all the other markers were expressed at higher levels in RIF, eMSC than in non-RIF cells although only HOXA11 and ITG $\beta 3$ showed statistically significant ($P < 0.05$) higher expression levels. Thus concluding that in this particular pilot study on determination of the expression levels of uterine receptivity markers in eMSC interestingly pointed that RIF and non-RIF eMSC were different regarding the expression of these markers. Future studies using these findings can brighten up more the role of eMSC in the endometrium receptivity and establishment of pregnancy [43].

Guo et al., tried to study, if Recurrent Implantation Failure (RIF) is associated with decreased expression of Platelet And Endothelial Cell Adhesion Molecule 1 (PECAM1) and Transforming Growth Factor β 1 (TGF- β 1) in the endometrium during the implantation window? As per this study it was shown that the expression of PECAM1 and TGF- β 1 is significantly decreased in the mid-secretory endometrium in women with RIF, which may account for embryo implantation failure. It is well established that RIF has become a significant determining factor that interferes with the improvement of pregnancy rates in IVF-embryo transfer (IVF-ET). The causes of RIF are complex and may involve the dysregulation of various growth factors, metabolites, and inflammatory cytokines. At present, the precise pathogenesis of RIF has not been elucidated. Hence they conducted a prospective case-control study. Endometrial tissue samples were obtained from January 2014 to December 2016 from two groups of women who had undergone IVF (RIF group, 22 women who underwent ≥ 3 ETs including a total of ≥ 4 good-quality embryos without pregnancy, control group, 18 women who conceived in their first treatment cycle). At the same time, samples were obtained from 18 women with infertility secondary to tubal factor in the early proliferative, late proliferative and mid-secretory phases of the menstrual cycle ($n = 6$ per group). Samples used for isolation of primary human endometrial epithelial cells and stromal cells (HEECs and HESCs) were collected in December 2017 from six women with infertility secondary to tubal factor. They evaluated gene expression using integrative whole genome expression microarray analysis, including differentially expressed gene screening, principal component analysis, and functional enrichment analysis. RT-qPCR, western blotting, immunohistochemistry, immunofluorescence co-localization analysis and short hairpin RNA (shRNA) plasmid transfection in Ishikawa cell line, HEECs and HESCs were used to investigate the expression of PECAM1 and TGF- β 1. On integrative data mining of whole-genome expression profiles identified cell adhesion as a key regulator in RIF. Database retrieval and literature review screened several novel cell adhesion-related genes that might participate in embryo implantation, which include PECAM1, Intercellular Adhesion Molecule 2 (ICAM2), Integrin Subunit β 2 (ITGB2), Selectin P (SELP) and TEK receptor Tyrosine Kinase (TEK). Among these targets, the mRNA and protein levels of PECAM1 were significantly lower in the RIF group than those in the control group. During the menstrual cycles of women with secondary infertility, the protein expression level of PECAM1 was the lowest in early proliferative phase, slightly increased in late proliferative phase and was the highest in mid-secretory phase. While the expression level of HOXA10, an endometrial receptivity marker, kept at a low level in early proliferative phase and increased in late proliferative phase, then maintained at a high level in the mid-secretory phase. Furthermore, TGF- β 1, mediated by PECAM1, was also decreased significantly in the RIF group. Using shRNA-based approach, we demonstrated that the depletion of PECAM1 significantly decreased the expression of TGF- β 1 in Ishikawa cells, as well as in primary HEECs and HESCs. These results indicated that PECAM1 and TGF- β 1 might play a pivotal role in modulating endometrial receptivity. Limitations of this study was that although they have shown that PECAM1 and TGF- β 1 were down-regulated in the women

with RIF, the molecular mechanism of the effect of the factors on the endometrial receptivity remain unclear. Their findings provide insight into the contribution of PECAM1 and TGF- β 1 in regulating implantation, which could be used to develop potential therapeutic methods for RIF [44].

Further Zhou et al., demonstrated impaired endometrial receptivity is one of the major causes of Recurrent Implantation Failure (RIF), although the underlying molecular mechanism has not been fully elucidated. In the present study, they demonstrated that Chromo Domain Y Like (CDYL) was highly expressed in the endometrium at mid-secretory phase during the normal menstrual cycles. However, the expression of CDYL was downregulated in the endometrial tissues obtained from women with RIF, consistently with the protein level of LIF, which is a marker of endometrial receptivity. In CDYL-knockdown human endometrial Ishikawa cells, we identified 1738 Differentially Expressed Genes (DEGs). Importantly, the Catenin Beta 1 (CTNNB1) expression was dramatically reduced responding to the CDYL inhibition, both in Ishikawa cells as well as the primary endometrial epithelial and stromal cells. In addition, the expression of CTNNB1 was decreased in the endometrium from RIF's patients as well. These results suggested that the expression of CTNNB1 was regulated by CDYL in endometrium. The cell migration was impaired by CDYL-knockdown in Ishikawa cells and primary Endometrial Stromal Cells (ESCs), which could be rescued by CDYL or CTNNB1 overexpression. Collectively, our findings indicated that the decreased expression of CDYL may suppress endometrial cell migration capability by affecting CTNNB1 expression, which would contribute to poor endometrial receptivity in women with RIF (Fig. 3-6) [45,46].

Figure 3: The expression of CDYL was significantly decreased in the endometrium of women with RIF. (A) The expression levels of CDYL in the control (n = 3) and RIF (n = 3) groups using microarray assay. (B) The mRNA levels of CDYL in the endometrium of the control (n = 22) and RIF (n = 22) groups. (C) Representative western blot analysis of CDYL and LIF in the control and RIF groups. (D) Densitometric quantification of CDYL in the

endometrium of the control (n = 22) and RIF (n = 22) groups. (E) Immunohistochemistry staining and (F,G) semi-quantification of CDYL protein expression in the control (n = 5) and RIF patients (n = 5). Bar = 50 μ m. (H) Receiver operating characteristic (ROC) curve plotting of the true positive vs. false positive rate, and the optimal cutoff value for endometrial issue for CDYL measurements. All data are presented as mean $\pm$ SD. * P < 0.05; ** P < 0.01; *** P < 0.001.

Figure 4: (A) SEM micrograph of endometrial surface from a patient with CE. Note the presence of thick mucus layer (right) containing debris and red blood cells. (B) Detail of Mucus and abundant bacteria are sticking on the microvilli and the cilia of the endometrial.

Figure 5: (A) Endometrial surface after oral treatment for CE. Note the presence of filamentous mucus (lower) and aggregations of red blood cells (upper middle). (B) Detail of Fibrinous material with red blood cells (middle) possibly emerging through diapedesis- and isolated bacteria (middle and lower right) are seen on the epithelium.

Figure 6: (A) Endometrial surface after intrauterine treatment for CE. The surface appears clean with abundant pinopodes. (B) Detail note the presence of fully developed pinopodes and of a small mucus aggregate (lower left).

Conclusion

Thus in cases of RIF, if antibiotics for CE fail despite intrauterine antibiotics (Fig. 4-6), then utilization of endometrial mesenchymal stem cells obtained from endometrial tissue or menstrual blood as in the 2 studies while in first one endometrial tissue mixed with PRP injected transmyometrially was efficacious in 23/29 cases of RIF at age >40 (average 41 years. in previous RIF) has been shown to be an innovative option in resistant refractory RIF. More evaluations have shown the involvement of Endometrial PECAM as well as TGF- β is selectively depressed in RIF cases along with alterations in CDYL and attempts to deeply study role of these molecules in implantation is warranted.

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