Regenerative therapy by endometrial mesenchymal stem cells in thin endometrium with repeated implantation failure. A novel strategy.

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ABSTRACT

Objective: Our primary objective was to evaluate the endometrial changes before and after transfer of endometrial mesenchymal stem cells (enMSCs) in a population of thinned endometrium with absence or hypo-responsiveness to estrogen and repeated implantation failure (RIF). The secondary objective was to evaluate the clinics results of the intervention in terms of clinical pregnancy (CP), early abortions pregnancy on going and live birth delivery rate (LBR) in in vitro fertilization (IVF) cycle.

Methods: A longitudinal and experimental study. The intervention was defined as "subendometrial inoculation of enMSCs," and the post-intervention changes were evaluated by the variables: endometrial thickness (Eth), endometrial flow cytometry (enFC), endometrial histopathology(enHP) and endometrial immunohistochemistry (IHQ). The variables were analyzed after the intervention (Post-treatment) regarding to previous values (Pretreatment).

Results: The comparison of Eth between pre and posttreatment with enMSCs leads to 5,24±1,24 mm vs 9,93±0,77 (p=0,000) respectively. Endometrial Flow Cytometry show significant differences in favor of Normalized variables in post-treatment, related with pretreatment, LT / Li, Lb / Li, NK / Li, CD8 / $CD3^+$ and CD4 / CD8 (p = 0.013, 0.002, 0.049, 0.000, 0.000) respectively. Only two variables Li / PC and CD4 / CD3 show NS (p = 0.167 and 0.118). Similar analysis is performed on enHP with an increase the normal HP in post-treatment (p=0.007). The CP rate was 79,31% (23/29), with a live birth delivery rate of % 45,45(10/22) and ongoing pregnancy 7/29 (24,14%).

Conclusion: It was noted a significative increase in endometrial thickness and normalize variables in enHP, enIHQ in post-treatment, previous, with enMSCs subendometrial inoculation. As a result, the IVF after treatment with enMSCs was a higher rate of CP and LBR.

Key Words: Endometrial stem cells, endometrial mesenchymal stem cells, thin endometrium, repeated implantation failure, endometrial flow cytometry, endometrial histopathology.

INTRODUCTION

One in nine couples in Europe and the USA is affected by implantation disorders and it is estimated that RIF has a prevalence of 15-20% in IVF (Teklenburg et al.,2010; Cicinelli et al.,2008).

There is enough evidence that appropriate Eth is essential for a successful pregnancy (Kovacs et al.,2003) and the presence of thin endometrium is related with low pregnancy (El-Toukhy et al.,2008; Glissant.,1985; Dickey et al.,1992). The optimal Eth for conception remains controversial among clinicians. Eth less than 7 mm on ultrasound is generally considered sub-optimal for embryo transfer (Eftekhar et al.,2018). Approximately 0,6-0,8% of patients do not reach minimum thickness to the embryo transference (Al-Ghamdi et al.,2008). It has been stated that the probably causes of thin endometrium are as follows, inflammatory causes (acute or chronic endometritis (CE)); iatrogenic (repeated curettage, polypectomy; hysteroscopic (myomectomy or laparoscopic) where the cavity is opened, and irrational use of clomiphene citrate (Mahajan δ Sharma.,2016). It is also indicated that thin endometrium may be a result of individual uterine structural pattern (Scioscia et al.,2009). In spite of the large variety of treatment, most of the choices achieve only minor modification in the endometrium thickness and have not been validated (Eftekhar et al.,2017). Angiogenesis is the formation of new blood vessels from existing vascular structures by elongation, intussusception or sprouting of endothelial cells and after birth the vascularization is determined and maintained by angiogenesis (Ahmed &El-Badri.,2017). Physiological angiogenesis does not occur in most organs in the adult. However, endometrium is the site where normal angiogenesis take place, and it is a fundamental process in the menstrual cycle as well as in embryo implantation (Gordon et al.,1995). Rogers and co-workers suggested that endometrial angiogenesis occurs by elongation and intussusception rather than sprout formation (Rogers et al., 1998). Blood vessels consist of an inner endothelial cell layer lining the vessel wall and perivascular pericytes, also known as mural cells, which envelop the vascular tube surface. Pericytes are multipotent cells that are heterogeneous in their origin, function, morphology and surface markers (Ahmed&El-Badri.,2017). Analysis of the anatomic relationship between pericytes and endothelial cells shows that they interact closely via juxtacrine or paracrine signaling (Gaengel et al.,2017). Pericytes were reported to respond to platelet derived growth factor receptor-β (PDGF-β) and transforming growth factor β (TGF-β) which are released by platelets after an injury (Mills et al.,2013). This chemotactic response to PDGF-β leads to the migration of pericytes to the outer layer of the blood vessel. This migration permits the endothelial cells proliferation at the wound site in response to vascular endothelial growth factor VEGF (Mohle et al.,1997). Angiogenesis plays a key role in the remodeling of the endometrium, being the vascular endothelial growth factor (VEGF) as an important regulator of this process. A number of works stated that VEGF is expressed differentially in the uterine with thin endometrium (Sugino et al.,2002; Kashida et al.,2001; Sharkey.,2000; Miwa et al 2009). The uterine NK cells (uNK) cells are a major source of cytokines and angiogenic growth factors, (Lash.,2006) including VEGF-A, placental growth factor (PLGF), and angiopoietin, (Trundley.,2004) which may produce cytokines to promote angiogenesis during embryo implantation. Although uNK cells counts are found be increased in women with recurrent miscarriage (RM) and RIF, angiogenesis seems to be paradoxical in the two groups of women, namely increased in RM and reduced in RIF (Chen et al.,2017). Previous research has shown that isolated CD56+ uNK cells from women with RIF produce lowest level of angiogenic factors, such as VEGF, PLGF, PDGF-BB, compared with women with RM and fertile controls (Chen et al.,2016). It has been proposed that the angiogenesis may be reduced by stimulating STAT5 pathway in uNK cells in women with RIF (Chen et al.,2017). There is sufficient

evidence of the existence of SCs in the human endometrium and the feasibility that they are a therapeutic resource in endometrial atrophy, thinned endometrium and Asherman syndrome (Singh et al.,2014). The skill to maintain a normal karyotype after several passages (Allickson et al.,2011), the ability to differentiate into multiple cell lines under standard culture (Rossignoli et al.,2013; Gargett et al.,2016), its immunosuppressive properties (inhibits LT, LB and NK) (Ribeiro et al.,2013), makes endometrial mesenchymal cells (enMSCs) a source of excellence in certain regenerative therapies. These immunomodulatory properties are explained by the release of inflammatory cytokines in the tissue (Bernardo.,2013). On the other hand, the low immunogenic capacity and tumorigenicity, makes it the choice in clinical application (Zhou et al.,2011). An important concept is that both infection and inflammation may inhibit regeneration of traumatized endometrium through damage to the stem/progenitor cells by effector molecules, which also contribute to the deposition of fibrotic tissue (Gargett δYe.,2012). Oocyte donation cycles are ideal to measure the independent effect of Eth as a parameter of endometrial receptivity because there is lower variability of embryo quality. RIF is a clinical entity which refers to a situation when implantation has repeatedly failed to reach a stage recognizable by pelvic sonography. It represents a very frustrating condition for both the professional and the patient. In order to isolate the endometrium as the main responsible for RIF, it was proposed, different embryonic condition and a number of consecutive infructuous IVF cycles. There is a tacit acceptance of defining RIF as the impossibility of obtaining clinical pregnancy after three consecutive IVF attempts, in which one to two embryos of high-grade quality are transferred in each cycle (Simon δ Laufer.,2012). The precise definition remains controversial, so other suggestions have been proposed (Polanski et al.,2014). Actually, the most accepted definition of RIF, it is a failure to achieve a clinical pregnancy after the transfer of three or more good-quality embryos in women < 35 years of age, and four or more good quality embryos in women ≥ 35 years during fresh or frozen embryo transfer cycles (Coughlan et al.,2014). In the presence of RIF of endometrial origin, the chronic endometritis (CE) and limited immunological alterations are plausible for specific treatments (Coughlan et al.,2014). In women with chronic endometritis (CE), the endometrial immune responses are often shifted towards pro-inflammatory profiles and consequently unfavorable to invading embryos (Park et al.,2016). The high association between Chronic Endometritis (CE) and RIF is established (14%-31%) as well as unknown etiology (28%) and recurrent pregnancy loss (9-13%) (Johnston-Mac Ananny et al.,2010, Cicinelli et al.,2005, Tersoglio et al.,2015, Bouet et al.,2016, Kotaro et al.,2017). A recent publication shows that 34.4% of women with RIF have CE, which is higher than those of women with recurrent pregnancy loss or fetal death (Wang et al 2019). The impact of chronic endometritis on perinatal outcomes has been considered, taking in to account CP and LBR with/without treatment (56vs7%) (McQueen et al.,2014). It has been proved that in CE had significantly decreased TGF-β and IL-10 expression in endometrium, which reflects T regulatory (Treg) cells numerical or functional deficiency (Wang et al.,2019). The resolution of the EC is of such complexity that the bacteriological negativization is inefficient as a criterion of cure, which could explain the high rate of persistent CE observed. (24.6 and 17.6%) (Cicinelli et al.,2015, Cicinelli et al.,2018). Our primary objective was to evaluate the endometrial changes before and after transfer of enMSCs in a population of thinned endometrium with absence or hypo-responsiveness to estrogen. The secondary objective was to evaluate the results of the intervention in terms of CP, early abortions, ongoing pregnancy and live births in IVF cycles.

METHODOLOGY

It is a longitudinal and experimental study focused on the endometrium (Salatino, 2018). The intervention was defined as "subendometrial inoculation of enMSCs" and the post-intervention changes were evaluated by the following variables: Eth (mm), enFC and enHP. The variables were analyzed after the intervention (Post-treatment) in reference to the previous values (Pretreatment). A Binomial distribution was obtained through the dichotomization of the enHP and enCF (Normal or Abnormal) variables, the latter in relation to the 95% CI values of reference group.

DEFINITION OF THINNED ENDOMETRIUM AND RIF

Thinned endometrium was considered to be the one that after twenty days of oestrogen supplementation with 8 mg/day dose of 17βestradiol which did not rich at least 7 mm of Eth measured in the midsagittal plane by transvaginal ultrasound (TVU). In ovulatory patients the endometrial thickness was observed on the day of LH and confirmed under the regimen described beforehand. We consider RIF as the absence of implantation after three or more cycles of IVF / ICSI or cryotransfer, where the cumulative number of embryos transferred was no less than three blastocysts with high quality 311-411 or 511 of Gardner-Schoolcraft, the latter under the criteria of Istanbul (Alpha Scientists in Reproductive and ESHRE special interest group of Embryology., 2012).

PATIENT SELECTION

We selected 29 patients with thin endometrium hypo-responsive/unresponsive to estrogens, with RIF. Inclusion criteria were absence of uterine malformation; autoimmune thyroid disease; thrombophilia; polyps; hydrosalpinx; and those who did not accomplish enHP, enIHQ and enFC pre/posttreatment; with the presence of normal uterine cavity ascertained by hysterosonography or hysteroscopy. In 23/29 (79,31%) were oocyte donation and 6/29 (20,69%) autologous cycles. The age of patients (year), number of previous IVF cycles, body mass index and length of sterility (year) were calculated with Mean±DS and (interval); 41,72±5,18 (32-53), 4,03±1,65 (3-8), 24,84±5,09 (19-40),13,66± 5,81 (5-20) respectively. The measure of basal Eth (mm) was 5,24±1,24 (2-6,9). In 6/29 (20.69%) they presented a history of uterine interventions (myomectomy=3, septum resection=1 and repetitive curettage=2). 6/29(20,69%) patients showed CE post medical history treated by specific antibiotics where was normalized the IMH. The basal pretreatment histopathology was normal in 6/29 (20,69%) and 23/29 (79,31%) presented abnormal patterns (Table 1).

Table 1 Demographic, Patient and basal clinical characteristics.

Number of cycles/patient	29
Number of previous IVF cycles	4,03±1,65 (3-8)
Body Mass Index (Kg/m^2)	24,84±5,09 (19-40)
Patient age (year)	41,72±5,18 (32-53)
Length of sterility (year)	13,66±5,81(5-20)
Basal endometrial thickness (mm)	5,24±1,24 (2–6,90)
Nº Patient w/ Previous Uterine Interventions	6/29 (20,69%)
Nº Patient w/ Previous CE by IHQ	8/29 (27,59%)
Oocyte donation cycles	23/29 (79,31%)
Homologous cycles	6/29 (20,69%)
Endometrial biopsy	

Normal	6/29 (20,69%)
Abnormal	23/29 (79,31%)

Note: Values were represented by mean ± DS. (Interval or %) where correspond.

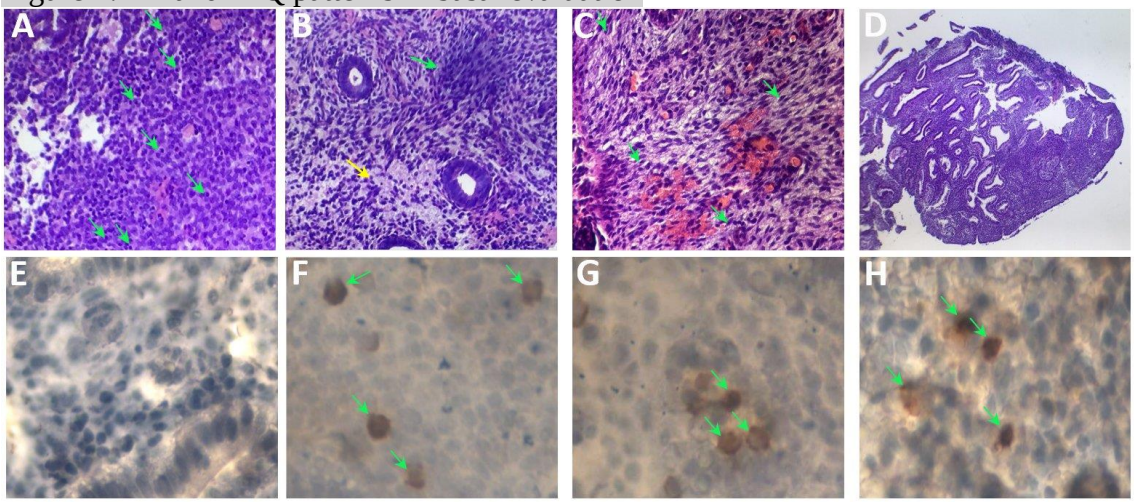
ENDOMETRIUM THICKNESS and ENDOMETRIAL BIOPSY

The endometrial thickness was measured in middle sagittal section by transvaginal sonography (TVS) with volumetric vaginal probe. The endometrial thickness in pre/pos-treatment was the highest value achieved under estrogen therapy on the day of the progesterone starting. In each patient, at least two endometrial biopsies were obtained, the first prior to treatment and the second after treatment with enMSCs. All biopsies were carried out under hormone replacement therapy (HRT) starting with 17β oestradiol 8mg/day by oral via, at least 20 days. The progesterone gel is added by vaginal via 180 mg/day dose. The biopsies were taken with the assistance of pipelle of Cornier with axial movements of the entire endometrial surface in order to ensure an ideal intake.

HISTOPATHOLOGICAL AND BACTERIOLOGICAL CRITERIA FOR NORMAL AND ABNORMAL ENDOMETRIUM

The histopathological criteria for dating were those corresponding to Noyes (Noyes and Hertig.,1950). In case of a CE history, the pretreatment biopsy must accomplish the bacteriological negativization with an endometrial stromal plasmacyte density index (ESPDI<0,25) as a criterion of cure (Akopians et al.,2015) (Kitaya et al.,2017). Being ESPDI the result from the sum of the stromal CD138 cell counts divided by the number of the HPFs evaluated. The following histopathological criteria were considered as abnormal endometrium: 1) Cumulous of lymphocytes, 2) presence of polymorphic inflammatory cells (stromal, superficial epithelium and intraglandular), 3) superficial mucosal edematous change, 4) presence of stromal cells like fibroblast (spindle stroma) (Check et al.,1991), 5) elevated density stromal cells, 6) pseudo-stratification and mitotic nuclei in both glandular and surface epithelial cells, 7) micro polyps, 8) delayed differentiation of endometrium (out of phase). The presence of no more than three of the previously established criteria was required as a posttreatment standardization one, as you can see figure 1.

Figure 1. HP and IHQ patterns in basal evaluation



The staining of tissue examples shown (**A**) Plasmatic Cells, (**B**) Stromal oedema and elevated Stromal density, (**C**) Spindle Stromal cells, (**D**) micropolyps, (**E**) Normal CD138, (**F**) High CD138, (**G**) High CD56 and (**H**) High CD20

The bacteriological examination consisted of fresh exam with Gram/Giemsa staining and culture on Thayer Martin medium, sheep blood agar, and chocolate agar, Agar medium Saboreaud, and EMB/CLDE of vaginal and endocervical samples and endometrium washing with PBS. Chlamydia trachomatis was tested by immunofluorescence, and Mycoplasma hominies and ureaplasma urealyticum by Mycofast-urea/arginine. All the cases included in the present study required negative bacteriology in pretreatment.

ENDOMETRIAL MULTICOLOUR FLOW CYTOMETRY

A FACS Calibur two lasers, four colors and six parameters (Bexton Dickinson®) was used for the cytometric evaluation. The following variables were considered: Li / PC (relation total lymphocyte / total cell population), ratio of T lymphocytes (LT), B lymphocytes (LB) and NK (NK) cells over Li; CD3+ CD4+ and CD3+ CD8+ in relation to Radio CD4 / CD8, and NK CD56 /CD16 subpopulations enNK. It was established a normal reference group for the cytometric variables (n = 25), corresponding to oocyte donors with a normal reproductive history (absence of abortions, with normal live births, no history of vaginosis, negative endometrial bacteriology and normal endometrial pathological anatomy) (Table 2). The values obtained in this study were considered for each variable as normal or abnormal related to the reference group.

Table 2. Endometrial Flow Cytometry. Normal Reference Value.

Variables - *n = 25*	**Mean ± 2SD**	**95% CI**	***P* value**
LT/Li	51,96 ± 16,05	35,908 - 68,01	0,112*
NK/Li	40,04 ± 14,58	25,458 - 54,62	0,157*
CD4/Li	44,32 ± 7,78	36,539 - 52,1	0,324*
CD8/Li	56 ± 12,31	43,6912 - 68,31	0,729*
CD4/CD8	0,64 ± 0,38	0,2676 - 1,01	0,137*
Li/Cells population	5.6‡	2 - 6,27	0.000†
LB/Li	2‡	1 - 2,98	0.000†

* p > 0,05 Normal distribution; † p < 0,05 No Normal distribution (by Shapiro Wilk test); CI = Confidence interval; SD = Standard deviation; ‡ Median

CELL ISOLATION and CULTURE

Endometrial tissue was dissociated using enzymatic and mechanical dissociation and separated into stromal single-cell suspensions and epithelial clumps as previously described with several modifications (Allickson et al.,2011, Akopians et al.,2015, Chen.,2016). Endometrial tissue samples were washed in DMEM/F-12 w/15mM HEPES buffer (Gibco, #11330-032), 5% new born calf serum (Invitrogen), and 1% antibiotic–antimycotic, (Gibco, # 15240-062) (Bench Medium), then weighed and cut into small pieces <1 mm3. The tissue fragments were digested with 0.5% (wt/vol) collagenase Type IV (Gibco, # 17104-019) and 40 μg/ml deoxyribonuclease type I (Worthington Biochemical Corporation) in DMEM/F-12 for 1.5 h at 37°C on a shaker. Typically, 10 ml of the cell dissociation medium was used per 1 g tissue. The dissociated cells were filtered through a sterile 40-μm cell strainer (BD Biosciences, Durham, NC, USA). Most

of the stromal cells and blood cells, present as a single- cell suspension, passed through the cell strainer into a sterile 50-ml polycarbonate tube, whereas the undigested fragments, mostly comprising glandular clumps, were retained on the strainer. Stromal single-cell suspensions were layered over Ficoll-Paque PLUS (GE Healthcare Bio-Sciences AB, #17-1440-02) and centrifuged to remove red blood cells. The medium/Ficoll interface, mainly containing stromal cells and peripheral blood mononuclear cells, was carefully aspirated, washed with Bench Medium, and then subjected to culture system. Endometrial stromal cells were seeded at a clonal density of 50-100 cells/cm^2 in twice flasks of 75 cm^2 and 25 cm^2 (Biofil#TCF 011250, TCF011050) with DMEM/F-12 plus wo/Hepes (GIBCO, 11320-033) supplemented with 10% new born calf serum (Invitrogen), 5% Anti-Anti (Gibco) and 2mM Glutamine (Invitrogen) up to obtain total confluence. When cells reached 75% confluence, they were detached using a 0,125% trypsin solution (GIBCO, #27250-018) diluted and plated into new T flasks. Cells were sub-cultured up to the third passage and cryopreserved (Amable et al.,2013).

IMMUNOPHENOTYPE OF enMSC CULTURE

In the confluent stage, the cells were characterized by flow cytometry previously marked with Human MSC analysis Kit (BD biosciences#562245) (CD19, CD34, CD45, CD73, CD90, CD105 y HLA-DR). Under the recommendations of International Society for Cellular Therapy, MSCs should be positive for CD73, CD90 and CD105, but be negative for CD34, CD45, CD11b or CD14, CD19 or CD79α, and HLA-DR. Additionally, SUSD2 / W5C5 and CD140b (PDFGF-Rβ^+) (BD biosciences, # 558821, 566657 respectively) were used as a positive marker. When the cell population reached the degree of confluence, usually in passage 5 to 7 and the results of the cytometry showed less than 0.3% for CD19, CD34, CD45 and HLA-DR, and more than 99.9% for the markers CD73, CD90 and CD105 were transferred under sonographic control with a transvaginal probe. In all cases, the transfer of enMSCs was carried out in the estrogenic phase under estrogen supplementation for 6 to 8 weeks.

CRYOPRESERVATION enMSCs

The residual flasks were cryopreserved in a suspension solution with 50% DMEM / F12 supplemented with 40% Fetal Bovine Serum (Internegocios,Argentina) and 10% DMSO, (MP Bio, USA). The cells suspended in the medium are frozen at -24 ° C for one hour and then at -70 ° C for 24 hours and finally transferred to the N2 tank in cryotubes. In the warm-up the tubes are transferred to a water bath at 37ºC and resuspended with DMEM / F12 solution with 10% FBS and centrifuged at 1200 rpm for 7 minutes. Viability was evaluated with 0.4% Tryptan Blue (SIGMA#T8154) in PBS.

PREPARATION PLATELET RICH PLASMA

The concentrate of plasma platelets obtained by centrifugation of the patient's whole blood was named PRP and this was applied as a diluent of the enMSCs. Peripheral blood was collected using tubes containing 3.2% sodium citrate solution. The preparation protocol was divided into two centrifugation steps. In the first centrifugation, the relative centrifugal force applied was 300 ×g for 5 min at 18 °C. The whole plasma above the buffy coat was collected (PRP1) and transferred to a new tube. The second centrifugation step used 700 ×g for 17 min at 18 °C. The platelet-poor plasma (PPP) was transferred to

a new tube. The platelet pellet obtained from 1 ml of PRP1 was resuspended in 300 µl of PPP (PRP2). Platelet activation was induced by adding 20 mM CaCl2 and 25 IU/ml human thrombin incubated at 37 °C for 1 h or at 4 °C for 16 h. Finally, for recovering the activated PRP2, samples were centrifuged at 3000 ×g for 20 min at 18 °C and the supernatant (activated PRP2) was collected by aspiration Activated PRP, hereafter referred as PRP, was frozen at -20ºC until use. (Amable et al.,2013a), (Amable et al.,2013b).

TRANSMIOMETRIAL TRANSFERENCE OF enMSC

Previously transfer the enMSCs were harvested using a 0.125% trypsin solution (GIBCO, #15090-046), washed with PBS and re-suspended in PBS containing 2% FBS. Viable and total cell numbers were determined using Trypan blue in Neubauer chambers. The enMSCs were transferred in a number of 2.5 to $3{,}6.10^6$ cells diluted in 1 ml of autologous PRP with the use of an Embryo Transfer Transmyometrial catheter (COOK # K-TTET-19-32.5) previous patient´s sedation.

EMBRYO LABORATORY, TRANSFER, RECEPTOR PROTOCOL

Briefly in presence of ovarian activity, it was performed hypothalamic suppression with the use of depot GnRHa, Triptorelin 3.76 mg in single dose, or Leuprolide acetate in doses of 200 to 300 micrograms / day, in long regime; beginning the estrogen replacement, in the presence of a plasmatic estradiol <30 pg / ml. Both the Estradiol valerate, as the 17 β-estradiol was administered in increasing doses, orally, according to the endometrial response and established protocol. Five or six days before the transfer was added Progesterone gel in daily doses 90 mg per vaginal via. In cases of inadequate endometrial response was associated with transdermal patches replacement with variable dose of (50-150 micrograms every 2.5 days). All transfer was individualized in according previous biopsy and level of IHQ hormonal receptors. Only expanded blastocysts of optimum quality (score 4.1.1 or 5.1.1) or hatched were transferred in a number no higher than two. In cases where a second blastocyst was of suboptimal quality (412, 421, 512 or 521) the transfer was made. In case of single embryo transfer (SET) was considered only optimal quality. All of the embryos at day 5 were cryopreserved and transferred at the time of optimization of the endometrium (Frozen-thawed embryo-transfer).

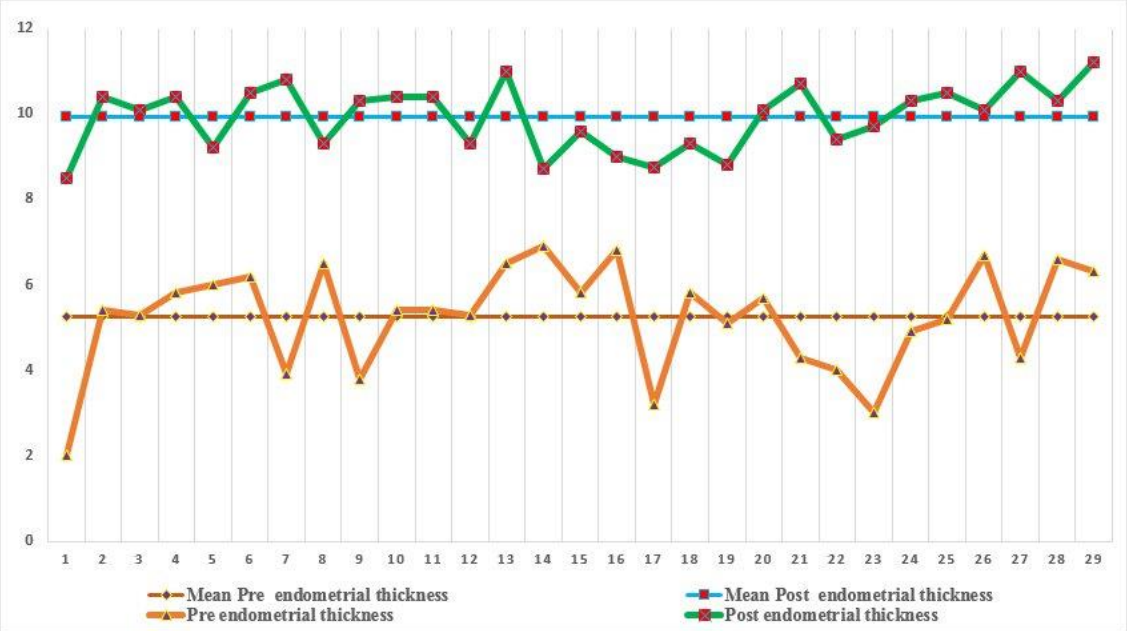
STATISTICAL ANALYSIS

Statistical analyses were performed with SPSS version 20 (IBM) and STATA statistical software version 14 (StataCorp). The data distribution was verified by the Shapiro-Wilk test. For the normal reference group, the 95% CI was calculated with the mean ± 2DS for normal distribution data; median and range for skewed data. The endometrial thickness was compared in the pre and post-treatment using the paired samples t-test; for the FC and HP the McNemar test was applied. The level of significance was set at $P<0.05$. In FC each variable was dichotomized considering the reference values and the histopathology was considered normal when ≤3 abnormal pattern was observed.

RESULTS

A total of 29 cycles/patients were analyzed with a mean age of 41,03±4,52, with a history of infertility of 13,66±5,81 years and 3,79±0,73 previous failed IVF cycles; all with thinned endometrium. As presented in table 3, the Eth between pre and posttreatment with enMSCs result 5,24±1,24 mm vs 9,93±0,77 (p=0,000) respectively. As display in Figure 2, the evolution of the endometrial thickness on a case-by-case basis is represented individually in relation to the intervention with enMSCs, ratifying an increase of more than 8 mm in all cases.

Figure 2. Individual evolution of endometrial thickness



As you can see in Table 3, after the dichotomization of the enFC variables (Normal or Abnormal) the lymphoid populations studied by FC show significant differences in favor of Normalized variables in post-treatment. As a result: LT / Li, LB / Li, NK / Li, CD8 / $CD3^+$ and CD4 / CD8 presented (p = 0.013, 0.002, 0.049, 0.000, 0.000) respectively. Both variables (Li / PC and CD4 / $CD3^+$) show a post-intervention increase but it turns out NS (p = 0.167 and 0.118). Analysis of enHP variables were carried out according to the criteria previously described. It was seen a normal increase in post-treatment (p = 0.007).

Table 3 Evolution of Endometrial Variables Pre and Post Treatment eMSCs

Variables	**Pre SCs**	**Post SCs**	***P* value**
Endometrial Thickness	5,25± 1,24	9,93 ± 0,77	p = 0,000*
Endometrial Flow Cytometry			
Li/PC			
Normal	13/29 (44,8%)	20/29 (69%)	p = 0,167†
Abnormal	16/29 (55,2%)	9/29 (31%)	
LT			
Normal	15/29 (51,7%)	26/29 (89,7%)	p = 0,013†
Abnormal	14/29 (48,3%)	3/29 (10,3%)	
LB			
Normal	8/29 (27,6%)	18/29 (62,1%)	p = 0,002†
Abnormal	21/29 (72,4%)	11/29 (37,9%)	
NK			
Normal	14/29 (48,3%)	23/29 (79,3%)	p = 0,049†
Abnormal	15/29 (51,7%)	6/29 (20,7%)	
CD4			
Normal	14/29 (48,3%)	21/29 (72,4%)	p = 0,118†
Abnormal	15/29 (51,7%)	8/29 (27,6%)	
CD8			
Normal	13/29 (44,8%)	25/29 (86,2%)	p = 0,000†
Abnormal	16/29 (55,2%)	4/29 (13,8%)	
CD4/CD8			
Normal	13/29 (44,8%)	28/29 (96,6%)	p = 0,000†
Abnormal	16/29 (55,2%)	1/29 (3,4%)	
Endometrial Histopathology			
Normal	6/29 (20,7%)	17/29 (58,6%)	p = 0,007†
Abnormal	23/29 (79,3%)	12/29 (41,4%)	

* T-test paired samples, † McNemar Test.

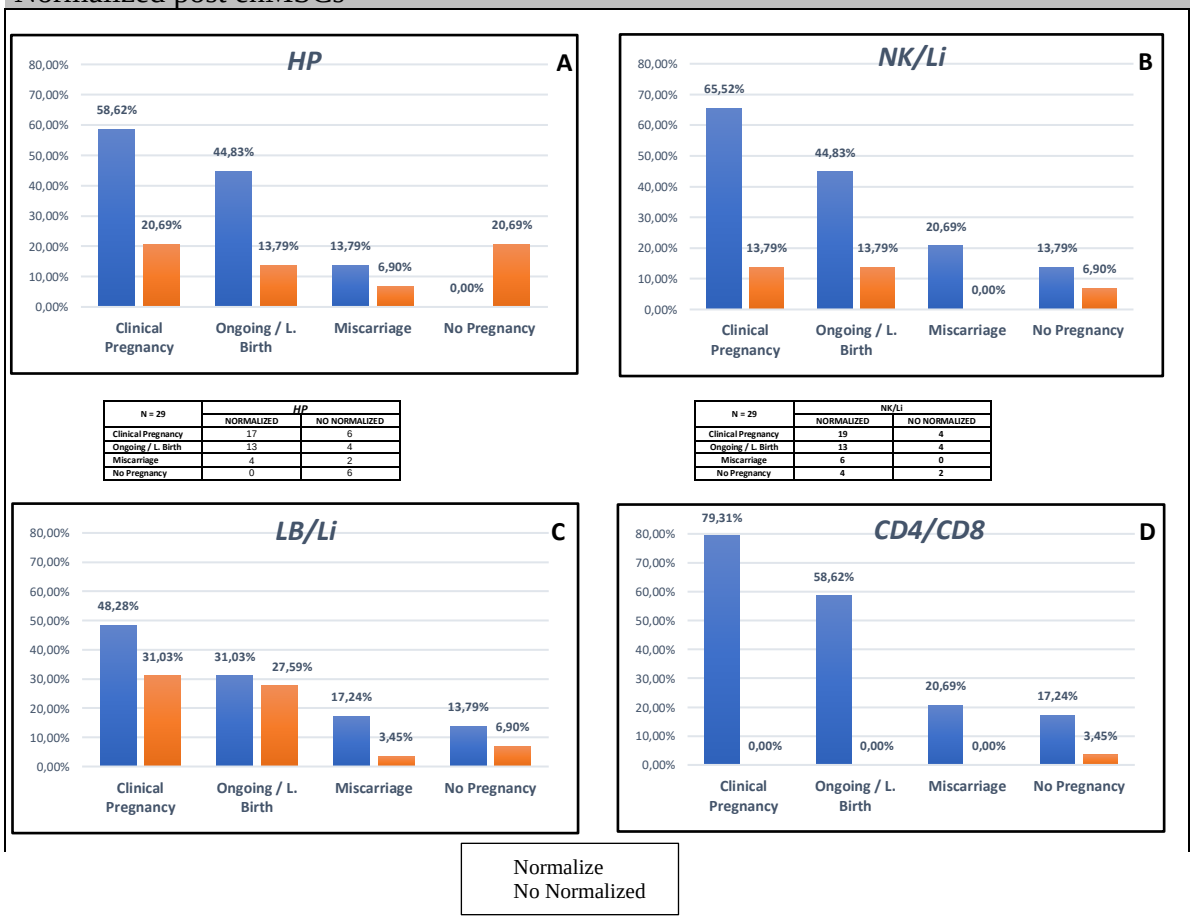
Are shown in table 4, over 29 females with embryo transfer, 23/29 (79%) they were clinical Pregnancy of which were born alive 10/22 (45,5%) and 7/29 (24,14) continue the evolution of their pregnancies with gestational ages greater than 17 weeks. The implantation rate results in 68,12% with a mean of 1,86±0,34 embryos transferred per patient. With a prematurity rate of 1/10 (10%) and single delivery 7/10 (70%).

Table 4 Clinical and Pregnancy Outcome	
n	29
Nº of Embryo Transferred (mean±SD)	1,86±0,34
Implantation Rate (%)	68,12%
Clinical Pregnancy (%)	23/29 (79,31%)
Abortion Rate (%)	6/29 (20,69%)
Ongoing Pregnancy (%)	7/29 (24,14%)
Live Birth Delivery Rate (%)*	10/22 (45,45%)
Single (%)	7/10 (70%)
Double (%)	3/10 (30%)
Premature Delivery	1/10 (10%)
Birth weight < 2500 gr	5
Birth weight > 2500 gr	8

* For Initiated Cycles

After treatment comparing the clinics outcome (CP, ongoing pregnancy/live birth, miscarriage and no pregnancy) in reference to normalization and no normalization (enHP, enNK, LB and CD4/CD8, are presented in figure 3.

Figure 3. Comparation of outcome pregnancies in relation HP and CF. Normalized or No Normalized post enMSCs



N = 29	LB/Li	
	NORMALIZED	NO NORMALIZED
Clinical Pregnancy	14	9
Ongoing / L. Birth	9	8
Miscarriage	5	1
No Pregnancy	4	2

N = 29	CD4/CD8	
	NORMALIZED	NO NORMALIZED
Clinical Pregnancy	23	0
Ongoing / L. Birth	17	0
Miscarriage	6	0
No Pregnancy	5	1

Comparation of (**A**) Histopatology, (**B**) enNK, (**C**) enLB and (**D**) enCD4/CD8 vs normalize or no normalized variables in posttreatment.

Discussion

Thin endometrium is a multifactorial condition, its management should be cause-related, with the aim of increasing endometrial receptivity and simplifying implantation (Levovitz δ Orviero., 2014). EnMSCs for their properties of high clonality, multipotentiality, regenerative capacity, immunomodulatory, angiogenic and low immunogenicity are proposed as an alternative in severe endometrial lesions. Adequate uterine vascularity and the regulating cells/factors are needed at the time of implantation, while inappropriate endometrial angiogenesis and immunity may lead to reproductive failure, in particular recurrent miscarriage and RIF (Chen et al.,2017). A number of not cellular treatments have been tried to increase endometrial development, but none has been validated up to now. Endometrial stem cell research is gaining momentum and the knowledge generated may be translated into the clinic within the next decade (Gargett and Ye.,2012). One of the points to be clarified is to define the cell line (s) that will be applied to the study, taking into account the limitations inherent in the method. The enSCs is a heterologous population that includes: mesenchymal stem cells (MSCs), epithelial stem cells (ESCs), endometrial side population (ESP) and endometrial regenerative cell (ERC) (Azizi et al.,2018). The Endometrial Side Population (ESP) are a mixed population, comprising predominantly of precursors of endothelial cells (Masuda et al.,2010) and epithelial and stromal cells (Cervelló et al.,2010). The proportion of SP cells in whole epithelial and stromal fractions fluctuate from 0.06-6,2% and 0,01-3,8%, respectively. The SP cells have the capability to extrude the DNA binding dye Hoechst 33342 via the ATP-binding cassette (Cervelló et al.,2010). Also, exhibits high clonogenicity (Tsuji.,2008), telomerase activity (Cervelló et al.,2011), but the most important and controversial issue is the heterogeneity of SP cells (Masuda et al.,2015). Specific markers of human endometrial MSC have been identified. Coexpression of CD140b (PDFGF-Rβ^{+}) platelet-derived growth factor-receptor beta (Schwab δ Gargett., 2007), and W5C5 has been used to isolate endometrial mesenchymal stem cells (Masuda et al., 2012). The gene profile of this CD 146 $^{+}$ PDFGF-Rβ^{+} population indicated that these cells expressed pericyte markers, and genes associated with angiogenesis/vasculogenesis, steroid hormone/hypoxia responses, inflammation, immunomodulation, and signaling pathways associated with MSC self-renewal and multipotency (Gargett δ Ye.,2012). Epithelial Progenitor cells (ESCs) is a small population with high proliferative potential that differentiated into large gland like structures (Nguyen et al.,2017). N-cadherin as a marker of epithelial progenitor cells can play a role in endometrial proliferative disorders like adenomyosis, endometriosis, and thin dysfunctional endometrium (Mills et al.,2013). The main limitation of EPCs recovery is there localization because they are placed near the basal gland fundus and closely to the myometrium. Because of that it is seldom observed in cells culture. Endometrial Regenerative Cells (ERC) have some features similar to MSC including the capability to modulate the immune system and stimulate Treg production. In this series, phenotyping indicates a predominance of MSCs, but probably due to sharing markers, the population has ESCs and ESP components. Actually, clinical application may not necessarily need a pure population of Stem Cells. However, for stem cell biology and understanding endometrial physiology, it is necessary to obtain single line cell (Masuda et al.,2015). It was established a normal reference group

for the cytometric variables (n = 25) in the absence of previous published dates. All the variables were analyzed in the normality of their distribution and 95% CI were calculated in order to increase the precision of data. A highly significant increase in endometrial thickness is observed after the inoculation of enMSCs expressing the high regenerative capacity of the intervention. In 8/29 (27,5%) of cases, they presented pretreatment values with critical values of <4 mm, despite having been subjected to estrogen therapy for over 20 days. It is interesting to note that in most of the previous IVF treatments the endometrium had not been isolated as the main cause of the repeated failures, even though the majority of the medical therapies had been tried (high doses of estradiol, HCG, aspirin, sildenafil, vitamin E and granulocyte colony stimulating factor). Normalization of the endometrial lymphoid population should be a primary objective in any treatment where the implantation conditions have been modified. Endometrial NK cells are the major leucocytes source present in the endometrium. It has been established that 47% of RIF presented uNK cell count outside the reference range, with a majority above the range and a smaller proportion below the range (Chen et al.,2017). In the present study, baseline FC showed in all cases a profound alteration of the lymphoid population and a significant normalization after enMSC. In this series a higher percentage of cytometric variables have been normalized, demonstrating the powerful modulator effect of enSCs. It is also interesting to see that in the subpopulation with CE background a high proportion shows alterations in the leukocyte population and the enMSCs were able to normalize 4/8 (50%); the totality of normalized cases resulted with live births. In contrast with the rest of CE no normalized after treatment with enMSCs, where 3/4 cases failed pregnancy. The special behavior of uNK and its implication in angiogenesis have been indicated in previous work, those who have shown that isolated $CD56^{+}$ uNK cells from women with RIF produce lowest level of angiogenic factors, such as vascular endothelial growth factor (VEGF), placental growth factor (PLGF), platelet-derived growth factor (PDGF-BB), compared with women with RM and fertile controls (Chen et al., 2016). The normalization impact of uNK and endometrial Treg is demonstrated when comparing normal and abnormal in relation to CP and evolutive pregnancy and LBR, (65,52 vs 13,79%, 44,83 vs 13,79% and 79,31 vs 0%, 58,62 vs 0%) respectively. The normalization of histopathology and immunohistochemistry in post-treatment with enMSCs results in higher clinical pregnancy in a population with repeated implantation failures what it represents a unique potential of MSCs in Assisted Reproduction. A high abortion rate (20.69%) is observed, which indicates that there are other factors to consider. This rate cannot be explained in reason of this variables. The strength of this work lies in the selection of the endometrium as an object of study and the measure the effects of the intervention in clinical results. The weakness of this paper is no achievement a complete immunophenotyping of enMSCs and no use of angiogenic markers. Even when the results are promising, more studies are needed to elucidate the possible mechanisms of endometrial therapy, optimize the technique and establish the biosecurity of it.

CONFLICT OF INTERESTS

No conflict of interest have been declared.

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References

Ahmed T.A., El-Badri N. Pericytes: The Role of Multipotent Stem Cells in Vascular Maintenance and Regenerative Medicine. In: Turksen K. (eds) Cell Biology and Translational Medicine, Volume 1. Advances in Experimental Medicine and Biology, vol 1079. Springer, Cham (2017). https://doi.org/10.1007/5584_2017_138

Akopians AL, Pisarska MD, Wang ET: The role of inflammatory pathways in implantation failure: chronic endometritis and hydrosalpinges. Semin Reprod Med. 2015; 33:298–304.

Al-Ghamdi A, Coskun S, Al-Hassn S,Al-RejjalR, Awartani K. The correlation between endometrial thickness and outcome of in vitro fertilization and embryo transfer(IVF-ET) outcome.Reprod Biol Endocrinol 2008;6:37.

Allickson JG, Sanchez A, Yefimenko N, et al. Recent studies assessing the proliferative capability of a novel adult stem cell identified in menstrual blood. Open Stem Cell J 2011;3:4–10.

Alpha Scientists in Reproductive and ESHRE special inter- est group of Embryology. The Istambul consensus work- shop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2012; 26:1270-83.

Amable PR, Carias RB, Teixeira MV, Pacheco I, Amaral RJF, Granjeiro JM, Borojevic R. Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. Stem Cell Research & Therapy 2013; 4:67.

Amable PR, Teixeira MVT, Carias RBV, Granjeiro JM, Borojevic R. (2013) Identification of Appropriate Reference Genes for Human Mesenchymal Cells during Expansion and Differentiation. PLoS ONE. 2013a; 8(9): e73792. doi:10.1371/journal.pone.0073792.

Azizi R, Aghebati-Maleki L, Nouri M, Marofi F, Negargar S, Yosefi M. Stem cell therapy in Asherman syndrome and thin endometrium: Stem Cell-based therapy. Biomed Pharmacother. 2018; 102:333-43. DOI.org/10.1016/j.biopha.2018.03.09.

Bayat N, Ebrahimibarough S, Ardakan MM, et al. Differ- entiation of human endometrial stem cells into schwann cells in fibrin hydrogel as 3D culture. Mol Neurobiol 2016; 53:7170–7176.

Bernardo ME, Fibbe WE. Mesenchymal stromal cells: Sensors and switchers of inflammation. Cell Stem Cell 2013;13:392–402.

Bouet PE, El Hachem H, Monceau E, Gariépy G, Kadoch IJ, Sylvestre C. Chronic endometritis in women with recurrent pregnancy loss and recurrent implantation failure: prevalence and role of office hysteroscopy and immunohistochemistry in diagnosis. Fertil Steril. 2016; 105:106-110.

Cervelló I, Gil-Sanchis C, Mas Aymara, Delgado-Rosas F, Martínez-Conejero JA et al. Human endometrial side population cells exhibit genotypic, phenotypic and functional

features of somatic stem cells. PloS ONE. 2010:5(6): e10964. doi:10.1371/journal.pone.0010964.

Check JH, Nowroozi K, Choe J, Dietterich C. Influence of endometrial thickness and echo patterns on pregnancy rates during in vitro fertilization, Fertil. Steril. 56 (6) (1991) 1173–1175.

Chen X, Liu Y, Jiang L, Wang CC, LiTC. Angiogenic cytokine profiles of human uterine CD56+ natural killer cells in women with recurrent reproductive failure. Am J Reprodt Immunol 2016;76(Suppl I):34.

Chen X, Man GCW, Liu Y, Wu F, Huang J, Li TC, Wang CC. Physiological and pathological angiogenesis in endometrium at the time of embryo implantation. Am J Reprod Immunol 2017;00e12693. https://doi.org/10.1111/aji.12693

Chen X, Mariee N, Jiang L, MPhil YL, Wang CC, Li TC,Laird S. Measurement of natural killer cell percentage in the periimplantation endometrium from fertile women and women with recurrent reproductive failure: establishment of a reference range. AmJObstetGynecol 2017;680e6. Doi.org/10.1016/j.ajog.2017.09.010.

Cicinelli E, De Ziegler D,Nicoletti R, Colagiglio G, Saliani NResta L, Rizzi D, De Vito D. Chronic endometritis correlation among hysteroscopic, histologic and bacteriologic findings in a prospective trial with 2190 consecutive office hysteroscopies. Fertil Steril. 2008; 89:677-84.

Cicinelli E, Matteo M, Tinelli R, Lepera A, Alfonso R, Indraccolo U, Marrochella S, Greco P, Resta L. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Hum Reprod. 2015; 30:323-330.

Cicinelli E, Matteo M, Trojano G et al. Chronic endometritis in patients with unexplained infertility: Prevalence and effects of antibiotic treatment on spontaneous conception. Am J Reprod Immunol.2018;79:e12782.

Cicinelli E, Resta L, Nicoletti R, Zappimbulso V, Tartagni M, Saliani N. Marrocchella S, Greco P, Resta L. Endometrial micropolyps at fluid hysteroscopy suggest the existence of chronic endometritis. Hum Reprod. 2005; 20:1386-9.

Coughlan C, Ledger W, Wang Q, et al. Recurrent implantation failure: definition and management. RBM Online. 2014; 28(1): 14-38.

Dickey RP, Olar TT, Curole DN, Taylor SN, Rye PH. Endometrial pattern and thickness associated with pregnancy outcome after assisted reproduction technologies, Hum. Reprod. 1992;7: 418–21.

Eftekhar M, Tabibnejad N, Tabatabaie AA. The thin endometrium in assisted reproductive technology: An ongoing challenge. Middle East Society Journal.2018;23:1-7.

El-Toukhy T, Coomararasamy A, Khairy M et al. The relationship between endometrial thickness and outcome of medicated frozen embryo replacement cycles. Fertil Steril 2008;89:832-839.

Gaengel K, Genove G, Armulik A, Betsholta C.Endothelial-mural cell signaling in vascular development and angiogenesis. Arterioscler Thromb Vas Biol .2003;29:630-38. Gargett CE, Schwab KE, Deane JA. Endometrial stem/ progenitor cells: The first 10 years. Hum Reprod Update 2016;22:137–163.

Gargett CE, Ye Louie. Endometrial reconstruction from stem cells. Fertil Steril.2012;98:11-20. doi:10.1016/j.fertstert.2012.05.004.

Glissant A, Mouzon J, Frydman,R. Ultrasound study of the endometrium during in vitro fertilization cycles, Fertil. Steril. 1985;44: 786–90.

Gonen Y, Casper RF, Jacobson W, Blankier J. Endometrial thickness and growth during ovarian stimulation: a possible predictor of implantation in in vitro fertilization, Fertil. Steril. 52 (3) (1989) 446–450.

Gordon JD, Shifren JL, Foulk RA, Taylor RN, Jaffe RB. Angiogenesis in the human female reproductive tract. Obstet Gynecol Surv.1995;50:688-97.

Johnston-Mac Ananny EB, Harnett J, Engmann LL, Nulsen JC, Sanders MM, Benadiva CA: Chronic endometritis is a frequent finding in women with recurrent implantation failure after in-vitro fertilization. Fertil Steril. 2010; 93:437-441.

Kashida S, Sugino N, Takiguchi S, Karube A, Takayama H, Yamagata Y, et al., Regulation and role of vascular endothelial growth factor in the corpus luteum during mid-pregnancy in rats, Biol. Reprod. 2001;64: 317–23.

Kitaya K, Matsubayashi H, Takaya Y, Nishiyama R, Yamaguchi K, Takeuchi T, Ishikawa T. Live birth rate following oral antibiotic treatment for chronic endometritis in infertile women with repeated implantation failure. Am J Reprod Immunol 2017:78: e12719.

Kitaya K, Matsubayashi H, Yamaguchi K, Nishiyama R, Takaya Y, Ishikawa T, Yasuo T, Yamada H. Chronic endometritis: potential cause of infertility and obstetric and neonatal complications. Am J Reprod Immunol 2016; 75:13-22.

Kotaro K, Matsubayashi H, Takaya Y, Nishiyama R, Yamaguchi K,Takeuchi T, Ishikawa T. Live birth rate following oral antibiotic treatment for chronic endometritis in infertile women with repeated implantation failure. Am J Reprod Immunol 2017; 78: e12719.

Kovacs P, Matyas S, Boda K, Kaali SG. The effect of endometrial thickness on IVF/ICSI outcome, Hum. Reprod. 2003;18: 2337–41.

Lash GE, Schiessl B, Kirkley M, et al. Expression of angiogenic growth factors by uterine natural killer cells during early pregnancy. J Leukoc Biol. 2006;3:572-580.

Lebovitz O δ Orvieto R, Treating patients with ‘‘thin” endometrium - an ongoing challenge, Gynecol. Endocrinol. (2014);30 (6): 409–414.

Mahajan N, Sharma S. The endometrium in assisted reproductive technology: How thin is thin?. J Hum Reprod Sci. 2016;9(1):3-8. Doi:10.4102/0974-1208. 178632.
Masuda H, Anwar SS, Bühring H.-J, Rao JR,Gargett CE. A novel marker of human endometrial mesenchymal stem-like cells, Cells Transpl. 2012; 21:2201-14. doi.org/10.3727/096368911X637362

Masuda H, Maruyama T, Gargett CE, Miyazaki K, Matsuzaki Y, Okano H, Tanaka M. Endometrial side population cells: potential adult stem/progenitor cells in endometrium. Biol Reprod. 2015; 93:1-8. doi:10.1095/biolreprod.115.131490.

Masuda H, Matsuzaki Y, Hiratsu E, Ono M, Nagashima T,Kajitani T et al. Stem cell-like properties of the endometrial side population: implication in endometrial regeneration. PloS ONE. 2010; 5(4): e10387. https://doi.org/10.1371/journal pone. 0010387

McQueen DB, Bernardi LA, Stephenson MD. Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise. Fertil Steril. 2014; 101:1026–30.
Mills SJ, Cowin AJ, Kaur P. Pericytes, mesenchymal stem cells and the wound healing process. Cells. 2013; 2:621-34.

Miwa I,Tamura H, Takasaki A, Yamagata Y, Shimamura K, SuginoN. Pathophysiologic features of ‘‘thin” endometrium, Fertil. Steril. 2009;91: 998–1004.

Mohle R, Green D, Moore MA, Nachman RL, Rafii S.Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. Proc Natl Acad Sci USA.1997;94:663-68.

Murakami K, Lee YH, Lucas ES, et al. Decidualization induces a secretome switch in perivascular niche cells of the human endometrium. Endocrinology 2014; 155:4542–4553.

Nguyen HP, Xiao L, Deane JA, Tan K-S, Cousins FL, Masuda H, et al. N- cadherin identifies human endometrial epithelial progenitor cells by in vitro stem cell assays. Hum. Reprod. 2017; 32 (11) 2254–2268.

Noyes R, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950; 1: 3-25
Polanski LT, Baumgarten MN, Quenby S, et al. What exactly do we mean by recurrent implantation failure? A systematic review and opinion. Reproductive Biomedicine Online. 2014; 28(4): 409-423.

Ribeiro A, Laranjeira P, Mendes S, et al. MSCs from umbilical cord matrix, adipose tissue and bone marrow exhibit different capability to suppress peripheral blood B, natural killer and T cells. Stem Cell Res Ther 2013;4:125.

Rogers PAW, Lederman F, Taylor N. Endometrial microvascular growth in normal and dysfunctional states. Hum Reprod Update.1998;4:503-8.
Rossignoli F, Caselli A, Grisendi G, et al. Isolation, characterization, and transduction of endometrial decidual tissue multipotent mesenchymal stromal/stem cells from menstrual blood. Biomed Res Int 2013;2013:901821.

Salatino, D. R. (2018). “Fundamentals of a new research method”. Inter. J. Res. Methodol. Soc. Sci., Vol., 4, No. 4: pp. x-xx. (Oct. – Dec. 2018); ISSN: 2415-0371 (In press).

Sharkey A.M, Day K, McPherson A, Malik S, Licence D, Smith SK, et al., Vascular endothelial growth factor expression in human endometrium is regulated by hypoxia, J. Clin. Endocrinol. Metab. 2000;85: 402–409.

Simon A and Laufer N. Repeated implantation failure: clinical approach. Fertil Steril 2012;97:1039-43.

Singh N, Mohanty S, Seth T, Shankar M, Bhaskaran S, Dharmendra S. Autologous stem cell transplantation in refractory Asherman’s syndrome: A novel cell based therapy. J Hum Reprod Sci.2014; 7:93-8. Doi: 10.4103/0974-1208.13886

Su K, Edwards SL, Tan KS, et al. Induction of endometrial MSCs into tissue-forming cells suitable for fascial repair. Acta Biomater 2014; 10:5012–5020.

Sugino N, Kashida S, Karube-Harada A,Takiguchi S, Kato H. Expression of vascular endothelial growth factor (VEGF) and its receptors in human endometrium throughout the menstrual cycle and in early pregnancy, Reproduction. 2002;123: 379–387.

Teklenburg G, Salker M, Heijnen C, Macklon NS, Brosens J.J.: The molecular basis of recurrent pregnancy loss: impaired natural embryo selection. Mol Hum Reprod. 2010; **16**:886–895.

Tersoglio AE, Salatino DR, Reinchisi G, Gonzalez A, Tersoglio S, Marlia C. Repeated implantation failure in oocyte donation. What to do to improve the endometrial receptivity? JBRA Assist Reprod. 2015; 19:44-52.

Trundley A, Moffett A. Human uterine leukocytes and pregnancy. Tissue Antigens. 2004;1:1-12.

Tsuji S, Yoshimoto M, Takahashi K, Noda Y, Nakahata T, Heike T. Side population cells contribute to the genesis of human endometrium. Fertil Steril. 2008; 90:1528-37. doi:10.1016/j.fertnstert.2007.08.005.

Wang WJ, Zhang H, Chen Z-q, Zhang W, Liu X-m, Fang J-y, Liu FJ,Kwak-Kim J. Reprod Biol and Endocrinol.2019;17:2. Doi.org/10.1186/s2958-018-0444-9.
Zhou C, Yang B, Tian Y, et al. Immunomodulatory effect of human umbilical cord Wharton’s jelly-derived mesenchymal stem cells on lymphocytes. Cell Immunol 2011;272:33–38.

Zuo W, Xie B, Li Ch, Yan Y, Zhang Y, Liu W, Huang J, Chen D. The Clinical Applications of Endometrial Mesenchymal Stem Cells. Biopreservation and Biobanking 2018;2:158-164. Doi:10.1089/bio.2017.0057