Italian Journal of
Gynæcology & Obstetrics
June 2019 - Vol. 31 - N. 2 - Quarterly - ISSN 2385 - 0868

Regenerative stem cell therapy in a infertile patient with atrophic endometrium. A case report.

Julia Kotlik[1], Maria Elena Mugavero[2], Kovpak Vitalii[1], Gaspare Cucinella[2], Antonio Perino[2]

[1]Biotexcom, Center for human reproduction, Kiev, Ukraine
[2]Department of Obstetrics and Gynecology Ospedali Riuniti Villa Sofia- V. Cervello. University of Palermo

ABSTRACT

Objective: In this case report, we describe a case of endometrial atrophy treated with adult autologous stem cells for mucosal regeneration that resulted in conception after in vitro fertilization-embryo transfer (IVF-ET).

Methods: A 36 years old patient was diagnosed with primary infertility for tubal factor (bilateral tubal blockage). This patient previously underwent an IVF procedure resulting in anembryonic gestation with subsequent curettage. The diagnostic hysteroscopy showed the presence of intrauterine adhesions which were lysed showing an atrophic endometrium. During the same procedure endometrium microfragments have been biopsied for eMSCs (Endometrial multipotent stromal cells) culturing. Six weeks later an IVF procedure with controlled ovarian stimulation has been carried out for the patient. On Day 4 of an estrogen supported cycle and after controlled endometrium microtraumatization , autologous cultured eMSCs autologous were released via flexible catheter, under ultrasonic guidance inside the uterine cavity of the patient.

Results: On Day 20 two frozen embryos at blastocyst stage at endometrium thickness of 8 mm have been transferred. After Day 30 post-transfer, a dichorionic twin pregnancy has been diagnosed in uterus by ultrasonic examination.

Conclusions: The endometrium quality is an important player in women fertility. Thus, the endometrial stem cell therapy can be used in females with intrauterine adhesions and atrophic endometrium , even though larger studies may be needed to establish this as proved line of treatment.

Keywords: thin endometrium, IVF, infertility, stem cell therapy, endometrial atrophy.

SOMMARIO

Scopo: In questo case report viene descritto il caso di una paziente con atrofia endometriale , trattata con cellule staminali autologhe per favorire la rigenerazione dell'endometrio. Questa terapia è esitata in un concepimento dopo fecondazione in vitro (IVF).

Metodi: La paziente è una donna di 36 anni con diagnosi di infertilità primaria da fattore tubarico (occlusione tubarica bilaterale). La stessa si è sottoposta in precedenza a una procedura di fecondazione in vitro esitata in una gravidanza anembrionata e quindi in successiva revisione di cavità uterina. L'isteroscopia diagnostica ha evidenziato la presenza di aderenze intrauterine che sono state lisate mostrando quindi un endometrio atrofico. Durante la stessa procedura, i microframmenti endometriali sono stati sottoposti a biopsia per la coltura di eMSC (cellule endometriali stromali multipotenti) . Sei settimane dopo è stata eseguita una procedura di IVF. Al quarto giorno di un ciclo mestruale, dopo una microtraumatizzazione controllata dell'endometrio, le eMSC autologhe in coltura sono state rilasciate tramite catetere flessibile, sotto guida ecografica, all'interno della cavità uterina.

Risultati: Al ventesimo giorno del ciclo sono stati trasferiti due embrioni congelati allo stadio di blastocisti (spessore dell'endometrio : 8 mm). Il trentesimo giorno post-transfer è stata diagnosticata ecograficamente una gravidanza gemellare bicoriale.

Conclusioni: La qualità endometriale è un fattore importante per la fertilità delle donne. Pertanto, la terapia con cellule staminali endometriali potrebbe essere utilizzata nelle donne con aderenze intrauterine ed endometrio atrofico, anche se sono necessari studi più ampi per stabilirla come vera e propria linea di trattamento.

Corresponding Author: Antonio Perino
antonio.perino@unipa.it
Copyright 2019, Partner-Graf srl, Prato
DOI: 10.14660/2385-0868-111

INTRODUCTION

The human endometrium is an highly regenerative organ undergoing over 400 cycles of shedding and regeneration during a women's lifespan[1–3]. Menstrual shedding, and the subsequent repair of the endometrial functionalis layer, is a process unique to humans and higher-order primates[4,5]. The endometrium regrows from a mere 1–2 mm thickness after menstrual shedding to 14 mm thickness in the secretory phase of the menstrual cycle[6], and is able to completely regenerate after parturition, and in post-menopausal (PM) women when exposed to oestrogen replacement therapy[2,7]. Human mesenchymal stem cells or multipotent stromal cells (MSC) have been identified in almost every adult tissue: bone marrow, adipose tissue,synovial membrane and the endometrium[8-10].

Endometrial MSCs (eMSC) were recently discovered and characterized in premenopausal endometrium where they are thought to regenerate the stromal vascular component of the functional layer each month[11]. Endometrial MSCs also possess a high capacity for proliferation and differentiation into mesodermal lineages and express characteristic MSC surface markers, fulfilling the minimal criteria for defining MSC[12].

Dysfunctional endometrial stem/progenitor cells may be responsible for the inability of some women to generate a sufficiently thick endometrium (7–8 mm) to support embryo implantation[13]. Thin dysfunctional endometrium unresponsive to estrogen stimulation is particularly challenging in In Vitro Fertilization (IVF) procedures. Thin endometrium is a clinical situation that makes the course of pregnancy difficult. The chances of getting pregnant with thin endometrium are minimal and even in case of pregnancy, there is a strong likelihood of miscarriage[14]. For these reasons it is recommended before IVF course to make some corrective procedures to facilitate an increase of endometrial thickness.

In this case report, we describe a case of endometrial atrophy treated with adult autologous stem cells for mucosal regeneration that resulted in conception after in vitro fertilization-embryo transfer (IVF-ET) .

CASE PRESENTATION

A 36 years old patient was diagnosed with primary infertility for tubal factor (bilateral tubal blockage). This patient previously underwent an IVF procedure resulting in anembryonic gestation with subsequent curettage. The consequent hypomenorrhoea has evolved on a background of significant endometrium hypoplasia. At the ultrasound examination an endometrial thickness of 3.5 mm on Day 18 of menstrual cycle (m.c.) was reported (**Figure 1**). The two following frozen embryo transfers did not lead to implantation, in spite of the long-term (over 6 months) endometrium conditioning with estrogens and gestagens.

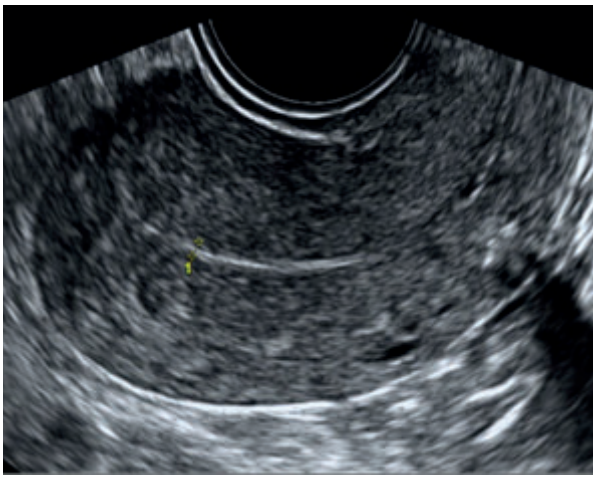

Figure 1

The diagnostic hysteroscopy showed the presence of filmy intrauterine adhesions due to the previous curettage, which were lysed showing an atrophic endometrium. During the same procedure endometrium microfragments have been biopsied for eMSCs culturing. Endometrial cells cultivation was performed by the methods already described by Lindenberg and Jividen[15-16]. Endometrial tissue was dissociated by enzyme treatment during 1 h in mixture of 0.05% collagenase IA and 0.05% pronase solution; the obtained cell suspensions were cultured in the DMEM:F12 medium with 10% human pooled serum, 2 мM L-glutamine and 1 ng/ml FGF-2 in the multi-gas incubator at 37°C, saturated humidity, 5% CO2 and 5% O2. The eMSCs were selected as adhesive fraction of cells attached to the culture plastic during 24 hours. The eMSCs were expanded during 2 passages; frequency of CFUf, phenotype and capacity for directed differentiation into adipocytes and osteoblasts were determined.

Six weeks later an IVF procedure with controlled ovarian stimulation has been carried out for the patient. The resulted embryos were vitrified at blastocyst stage. On Day 4 of an estrogen supported cycle and after controlled endometrium microtraumatization (to improve the grafted cells

adhesion) with an outer sheath of flexible catheter for embryo transfer, 2×107 autologous cultured eMSCs (CD73+CD90+CD105+CD49f-CD34-CD45-) in 1 ml of autologous PRP were released via flexible catheter under ultrasonic guidance inside te uterine cavity of the patient.

At ultrasonic examination on Day 13 m.c. the endometrium thickness was measured as high as 8 mm after cultured EndSCs grafting (**Figure 2**).

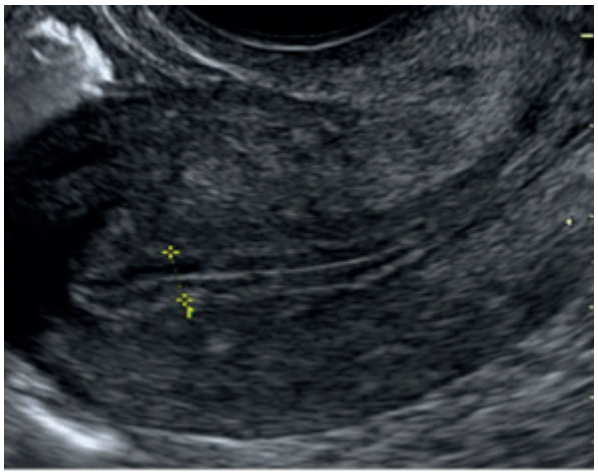

Figure 2

It was established a support for the second phase of cycle by gestagens. On Day 20 m.c. two frozen embryos at blastocyst stage at endometrium thickness of 8 mm have been transferred. At Day 13 post-transfer the hCG level increased up to 120 mIU/ml. After Day 30 post-transfer, a dichorionic twin pregnancy has been diagnosed in uterus by ultrasonic examination (**Figure 3**).

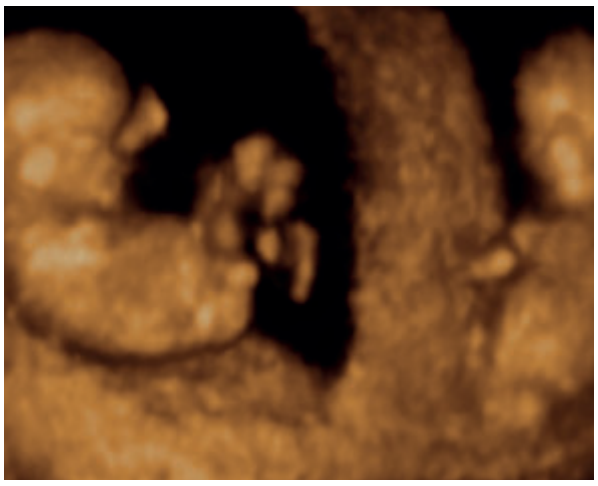

Figure 3

The course of pregnancy was regular until the 31st week in which there was a premature discharge of amniotic fluid. A caesarean section was performed: male baby weight was 1630 g (Apgar 7 /8), female baby weight was 1400g (Apgar 6/8). After 4 days of intensive therapy they were on a second stage of therapy When both babies gained weight, they were discharged . Three years after the procedure the patient has regular cycles of 27-28 days, and normal menstruation lasting 5 days

DISCUSSION

Prianishnikov was the first to consider the existence of endometrial adult stem cells (ASCs) and, in 1978, he proposed ASCs to reside in the deeper basalis layer, with their differentiation marked by functional changes (acquiring) in hormonal receptivity[14]. This author suggested a hierarchical hormone receptiveness in endometrial cells, matching their level of maturity, and therefore, the most primitive hormone-independent ASCs initially differentiate first into oestrogen-dependent cells, and then they may further differentiate into both oestrogen and progesterone-dependent cell. Terminally-differentiated cells were expected to be only progesterone-dependent, and were postulated to have a limited lifespan[17] . Endometrial epithelial cells in particular are difficult to culture in vitro for long duration; indeed the in vivo phenotype cannot be maintained using traditional 2D culture methods. For this reason, functional assays that have been developed to examine the stem cell properties in vitro, may not be suitable for endometrial epithelial cells. Furthermore, the true and conclusive confirmation of an endometrial epithelial stem cell requires the demonstration that they are able to produce all of the epithelial cell types that exist in all regions of the endometrium [23]

Endometrial atrophy (EA) is a clinical condition in which the endometrium is too thin and never grows more than 5 mm thickness[18]. The incidence of thin endometrium in ART has been reported to be between 1.5 and 9.1% [21]. Both pathological conditions share a common clinical effect: the absence of functional endometrium. Stem cell therapy targeting the endometrial niche with the ultimate aim of replenishing the cellular compartment of the functionalist layer offers a promising possibility for treating AS and EA.[24]

Cells derived from the bone marrow expressing CD133/VEGFR2 represent a subpopulation of cells, with endothelial progenitor capacity ; these cells are known as Endothelial progenitor cells (EPCs)[19], that can be mobilized to the circulation and can improve

neoangiogenesis afforded by pre-existing endothelium. CD133+ bone marrow-derived stem cells (BMDSCs) regenerate vascularization and induce endometrial proliferation leading to the creation of an autologous reconstruction of the endometrium. Probably the mechanism involved is that engrafted CD133+ BMDSCs induce the secretion of the paracrine factors thrombospondin 1 and insulin-like growth factor, which activate mitosis of surrounding cells, thus inducing endometrial regeneration.[24]

The Endometrial Mesenchymal stem cells (eMSCs) are multipotent, highly proliferative, self-renewing adult stromal stem cells found in a perivascular location in the endometrium and distinct from endometrial stromal fibroblasts [22]. They also expresses genes involved in steroid hormone and hypoxia responses, inflammation, immunomodulation and cell communication, emphasizing their role in tissue homeostasis and immune tolerance required for embryo implantation and placental development. As described for other organs, eMSCs predominantly reside in the perivascular niche of both the basal and functional layer. The ease with which endometrial tissue can be obtained by pipelle biopsy without anaesthetic in comparison to the collection of bone marrow aspirates or adipose tissue liposuctions makes it an attractive source of MSCs for regenerative medicine[21]. Menstrual blood is an even easier source for collection of MSC-like cells, although greater attention is required to ensure sterility of the cell product and methods for the purification of eMSCs from this source have not been refined.

CONCLUSIONS

The properties of eMSCs are favourable for further development in regenerative medicine applications as they are relatively easy to obtain compared with the current commonly used sources. The endometrium is one of the few tissues where MSC can be obtained without invasive and painful interventions eventually requiring anesthesia

The endometrium quality is an important player in women fertility. An insufficiently trophic endometrium may result in sub-fertility and failed IVF procedures . Thus, the endometrial stem cell therapy can be used in females with intrauterine adhesions and atrophic endometrium , even though larger studies may be needed to establish this as proved line of treatment.

REFERENCES

1) Cervello, I.; Gil-Sanchis, C.; Mas, A.; Delgado-Rosas, F.; Martinez-Conejero, J.A.; Galan, A.; Martinez-Romero, A.; Martinez, S.; Navarro, I.; Ferro, J.; et al. **Human endometrial side population cells exhibit genotypic, phenotypic and functional features of somatic stem cells**. PLoS ONE 2010, 5, e10964.

2) Gargett, C.E. Uterine stem cells: **What is the evidence?** Hum. Reprod. Update 2007, 13, 87–101.

3) Jabbour, H.N.; Kelly, R.W.; Fraser, H.M.; Critchley, H.O. **Endocrine regulation of menstruation**. Endocr. Rev. 2006, 27, 17–46

4) Brenner, R.M.; Slayden, O.D. **Molecular and functional aspects of menstruation in the macaque**. Rev. Endocr. Metab. Disord. 2012, 13, 309–318.

5) Slayden, O.D.; Brenner, R.M. **A critical period of progesterone withdrawal precedes menstruation in macaques**. Reprod. Biol. Endocrinol. 2006, 4 (Suppl. 1), S6.

6) Fleischer, A.C. **Sonographic assessment of endometrial disorders**. Semin. Ultrasound CT MR 1999, 20, 259–266.

7)Gargett, C.E. **Identification and characterisation of human endometrial stem/progenitor cells**. Aust. N. Z. J. Obstet. Gynaecol. 2006, 46, 250–253

8) Silva Meirelles L, Chagastelles PC, Nardi NB. **Mesenchymal stem cells reside in virtually all post-natal organs and tissues**. J Cell Sci 2006;119(Pt 11):2204 – 2213

9) Beltrami AR, Cesselli D, Bergamin N, Marcon P, Rigo S, Puppato E, D'Aurizio F, Verardo R, Piazza S, Pignatelli A et al. **Multipotent cells can be generated in vitro from several adult human organs (heart, liver, and bone marrow)**. Blood 2007;110:3438–3446.

10) Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L et al. **A perivascular origin for mesenchymal stem cells in multiple human organs**. Cell Stem Cell 2008;3:301 – 313.

11) Gargett CE, Schwab KE, Zillwood RM, Nguyen HP, Wu D. **Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium.** Biol Reprod 2009;80:1136–1145

12) Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. **Minimal criteria for defining multipotent mesenchymal stromal cells**. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–317.

13) Yu D, Wong YM, Cheong Y, Xia E, Li TC. **Asherman**

syndrome—one century later. Fertil Steril 2008;89:759 – 779
14) Ohno Y, Fujimoto Y. **Endometrial oestrogen and progesterone receptors and their relationship to sonographic appearance of the endometrium**. Hum Reprod Update. 1998;4:560–4
15) S. Lindenberg at al. **Isolation and culture of human endometrial cells** - Fertil.Steril. - Vol.41,No 4, April 1984. - h.650-652
16) K. Jividen, V.J. Movassagh, A. Jazaeri, H.i Li :**Two Methods for Establishing Primary Human Endometrial Stromal Cells from Hysterectomy Specimens**.- J Vis Exp. - 2014. - Vol 87. - 51513. doi :10.3791/51513
17) Prianishnikov, V.A. **A functional model of the structure of the epithelium of normal, hyperplastic and malignant human endometrium: A review**. Gynecol. Oncol. 1978, 6, 420–428
18) Senturk LM, Erel CT. **Thin endometrium in assisted reproductive technology**. Curr Opin Obstet Gynecol 2008;20:221–228.
19) Urbich C, Dimmeler S. **Endothelial progenitor cells: characterization and role in vascular biology**. Circ Res 2004;95:343 – 353.
20) Ulrich D, Muralitharan R, Gargett CE. **Toward the use of endometrial and menstrual blood mesenchymal stem cells for cell-based therapies**. Expert Opin Biol Ther 2013; 13:1387 – 1400
21) Bu Z, Sun Y. **The impact of endometrial thickness on the day of human chorionic gonadotrophin (hCG) administration on ongoing pregnancy rate in patients with different ovarian response**. PLoS One 2015;10: e0145703
22) Caroline E. Gargett, Kjiana E. Schwab, and James A. Deane. **Endometrial stem/progenitor cells: the first 10 years** . Human Reproduction Update, Vol.22, No.2 pp. 137–163, 2016
23) Nicol Tempest, Alison Maclean and Dharani K. Hapangama. **Endometrial Stem Cell Markers: Current Concepts and Unresolved Questions** . *Int. J. Mol. Sci.* 2018, 19, 3240;
24) Xavier Santamaria, Sergio Cabanillas, Irene Cervello, Cristina Arbona, Francisco Raga, Jaime Ferro, Julio Palmero, Jose Remoh 1, Antonio Pellicer, and Carlos Simon. **Autologous cell therapy with CD1331 bone marrow-derived stem cells for refractory Asherman's syndrome and endometrial atrophy: a pilot cohort study**. Human Reproduction, Vol.31, No.5 pp. 1087–1096, 2016