CASE REPORT

Granulocyte colony-stimulating factor (G-CSF): a mediator in endometrial receptivity for a patient with polycystic ovary (PCO) undergoing in vitro maturation (IVM)

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SUMMARY

Proliferative and secretory changes at the endometrial lining are the result of a complex intrauterine environment where sex steroid hormones and different local factors play an important role for endometrial thickening. Optimal endometrial thickness reflects an adequate maturation which is a key factor for embryo implantation. Here, we present a case of a woman with polycystic ovary who was treated using in vitro maturation (IVM) techniques. In addition, this patient showed a dyssynchrony between the endometrial phase characterised by endometrial thinning and the embryo development which had a negative impact for embryo implantation. A protocol using uterine perfusion of granulocyte colony-stimulating factor (G-CSF) was performed as an alternative treatment for the unresponsive endometrium. We found that uterine infusion of G-CSF quickly increased endometrial thickness resulting in a successful pregnancy and healthy born baby. These results suggest that G-CSF is a factor that participates during endometrial remodelling enhancing the synchronisation between uterine environment and embryo development.

BACKGROUND

Adequate proliferative and secretory changes of the endometrium characterised by modification of stromal cells, increase of uterine glands and vessels and attraction of uterine immune cells are indispensable to ensure a successful implantation of a healthy blastocyst. The development of a receptive endometrium is a consequence of a coordinated interplay among steroid hormonal stimuli, cytokines and biological interactions that allow an optimal thickness of the endometrium to achieve the successful progression of pregnancy.¹ In this scenario, granulocyte colony-stimulating factor (G-CSF) is a haematopoietic cytokine actively produced in the maternofetal interface during embryo implantation and early pregnancy by the decidual and placental tissues. This cytokine is necessary for mobilisation and recruitment of lymphocytes, uterine dendritic cells, and macrophages playing a crucial role as an endometrial remodelling factor during the mid-secretory phase of the menstrual cycle (days 19–23 of a normalised 28-day cycle) known as window implantation.² Thus, it is thought that G-CSF prepares the endometrium in only a few days to suppress any immune aggression

to the presence of an embryo allowing an adequate endometrial receptivity.³

Non-stimulated cycles combined with IVM of oocytes are practical treatments for women with different causes of infertility such as PCO.^{4–5} These patients show a deficient developmental capacity of immature oocytes that depends on the follicular size, concentrations of intrafollicular hormones and different ovarian growth factors.^{6–7} Consequently, embryo implantation depends on both the quality of the embryo and the receptivity of endometrium mainly marked by the proportion and number of endometrial immune cells. The dyssynchrony between the in vitro matured embryos and the satisfactory development of the endometrium might be the cause of low implantation success in PCO patients who underwent assisted reproductive techniques (ART).^{8–9} PCO patients show poor endometrial quality reflected on inadequate endometrial thickness giving rise to the development of protocols for endometrial preparation. Several studies report that G-CSF instillation offered to patients with severe fertility disorders related to embryo implantation failures such as repeated pregnancy loss or unsuccessful ART cycles, improved pregnancy rates to 26%.^{3, 10–14} Our aim on this IVM cycle is to achieve a perfect synchronisation between the receptive endometrium and the development of embryo. We report the use of G-CSF as a factor for quick endometrial remodelling during IVM cycles. Furthermore, improving unresponsive thin endometrium by uterine instillation of G-CSF has not previously been reported in this setting making this an interesting case.

CASE PRESENTATION

In May 2011, a 25-year-old Caucasian woman came to our clinic with a medical history of secondary infertility due to three consecutive pregnancy losses. These pregnancies consisted of comprising two previous ectopic pregnancies in 2009 (1 in each tube) and one spontaneous miscarriage in 2010. Ectopic pregnancies were treated by right salpingectomy via laparoscopy and she received a dose of methotrexate. As a relevant personal history she had polycystic ovaries and we confirmed by transvaginal pelvic ultrasound that both ovaries contained more than 12 small follicles (<10 mm) in each gonad. After detailed medical counselling our patient decided to undertake natural cycle combined with IVM of

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oocytes. At the ninth day of the natural cycle, oocyte retrieval was made obtaining a total number of 18 immature oocytes that were matured in vitro. We achieve 10 oocytes at the stage of Meiosis II, 3 oocytes at Meiosis I, 3 oocytes at prophase I and 2 oocytes at the atretic stage after 24 h of IVF. At the same day of oocyte retrieval the endometrial thickness was measured (5.7 mm) and we immediately instilled 1 ml (30 MU equivalent to 300 µg of filgrastim) of G-CSF (Neupogen) into the uterine cavity. Matured oocytes (total five) were injected with sperm from the partner and three embryos were transferred at 72 h of incubation. At that time endometrial thickness improved to 8.9 mm. Fourteen days later after embryo transfer βhCG was detected and ultrasonography at 21 days confirmed a viable singleton intrauterine pregnancy. Sonography at 11 and 24 weeks showed adequately fetal growth.

OUTCOME AND FOLLOW-UP

Our patient had an uncomplicated antenatal course and she had an uncomplicated caesarean-section delivery of a healthy girl weighing 3.060 kg and 49.5 cm in height.

DISCUSSION

Endometrial receptivity is characterised by local changes such as increased secretions of endometrial glands, alteration of cell surface adhesion molecules and differentiation of fibroblast in the periarterial regions (decidualisation) into functionally distinct decidual cells. A mathematic calculation estimated that normally endometrial receptivity accounts for about 31–64% of successfully

embryo implantation.^{15–17} The only known marker for uterine receptivity in humans is implantation.⁸ The decidua plays a key role in trophoblast invasion and is tightly related with numerous immunological factors that are produced and regulated locally giving rise to a perfect immunoregulation of different populations of immune cells (eg, T-helper cell population: Th-1 and Th-2) in the fetal maternal interface resulting in a successful embryo implantation. Nowadays, we know that the Th-2 cell population predominates in the basal plate region of the placenta, blocking any maternal Th-1 cell attacks against the embryo and thereby preventing loss of pregnancy.¹⁸ G-CSF is a maternal immunological factor that induces Th-2 immune response by increasing secretion of chemokines such as interleukin-4, interleukin-5, interleukin-10 and transforming growth factor-β, as a part of endometrial maturation during embryo implantation.¹⁹

Retardation of endometrial immunoregulation is related to inadequate hormonal stimulation by endocrine ovarian failure resulting in a weakly proliferative and unresponsive endometrium (figures 1 and 2). Patients with PCO show impaired developmental capacity of in vivo maturation of oocytes associated with poor endometrial quality reflected failing to improve endometrial thickness due to low synthesis of steroid hormones.^{7 20} Therefore, studies suggest that an endometrial thickness of more than 8 mm is preferable to maximise embryo implantation rates.^{3 10 21} Women, such as our patient, with two prior embryo transfers with documented inadequate endometrial thickness have very low chances of achieving a successful pregnancy. Remarkably, quick endometrial improvement was established

Figure 1 Human endometrial receptivity. (A) Normal endometrial receptivity: endometrium proliferates under oestrogen enhancement and progesterone from the luteinised follicle leads to endometrial differentiation, vascular remodelling by action of cytokines such as granulocyte colony-stimulating factor (G-CSF) that attract leucocytes to start decidualisation and ensure a receptive endometrium for successful implantation. (B) Non-receptive endometrium: in patients with polycystic ovary/polycystic ovarian syndrome, steroid receptor and functional factors such as G-CSF appear to be altered indicating altered endometrial receptivity and impaired embryoendometrial synchronisation leading to a recurrent implantation failure. (C) Growth factors such as G-CSF is a mediator of endometrial receptivity improvement: exogenous growth factor recovery the synchrony through the leucocytes migration ensuring vascular remodelling, endometrial proliferation, decidualisation and uterine gland transformation to achieve embryo implantation, placentation and early pregnancy maintenance. E2, oestrogen; P4, progesterone.

Figure 2 Anatomical and functional changes predominantly hormones and growth factors expression, and leucocytes during endometrial secretory phase. Achieving the optimal endometrial receptivity to ensure the successful embryo implantation.

after intracavitary instillation of G-CSF that induced a local proliferative effect on endometrial lining as a signal of endometrial receptivity and synchronisation between embryo and endometrium during the narrow window of implantation.

G-CSF instillation has been offered to patients with severe fertility disorders to improve pregnancy rates around 26%.^{3 10–14} Consequently in our case report, non-stimulated cycle combined with IVM and G-CSF instillation might be a beneficial treatment in patients with history of embryo implantation failure, repeated pregnancy loss or failure in multiple ART cycles. To the best of our knowledge this report establishes for the first time that endometrial dyssynchrony present in IVM cycles can be quickly improved by G-CSF local endometrial perfusion to achieve implantation and successful pregnancy. The study also shows that G-CSF during implantation, via endometrial perfusion, improves the immunological endometrial receptivity providing a treatment option to other infertile patients that show inadequate or unresponsive endometrial proliferation during ART cycles. Further studies are required to accurately elucidate the link between endometrial G-CSF perfusion and IVM.

Learning points

- ▶ Granulocyte colony-stimulating factor local perfusion is a novel intervention for quick endometrial remodelling in embryo implantation on in-vitro maturation cycles.
- ▶ Local uterine perfusion of granulocyte colony-stimulating factor improves endometrial receptivity ensuring positive outcome in embryo implantation, successful pregnancy, and healthy baby at home.

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Competing interests None.

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