



INTERNATIONAL CONFERENCE ON PARADIGM CHANGES
in the Management of Early Pregnancy and Pregnancy Loss (PCMEP)
London, UK, February 21-24, 2019

THE ROLE OF FILGRASTIM IN RPL

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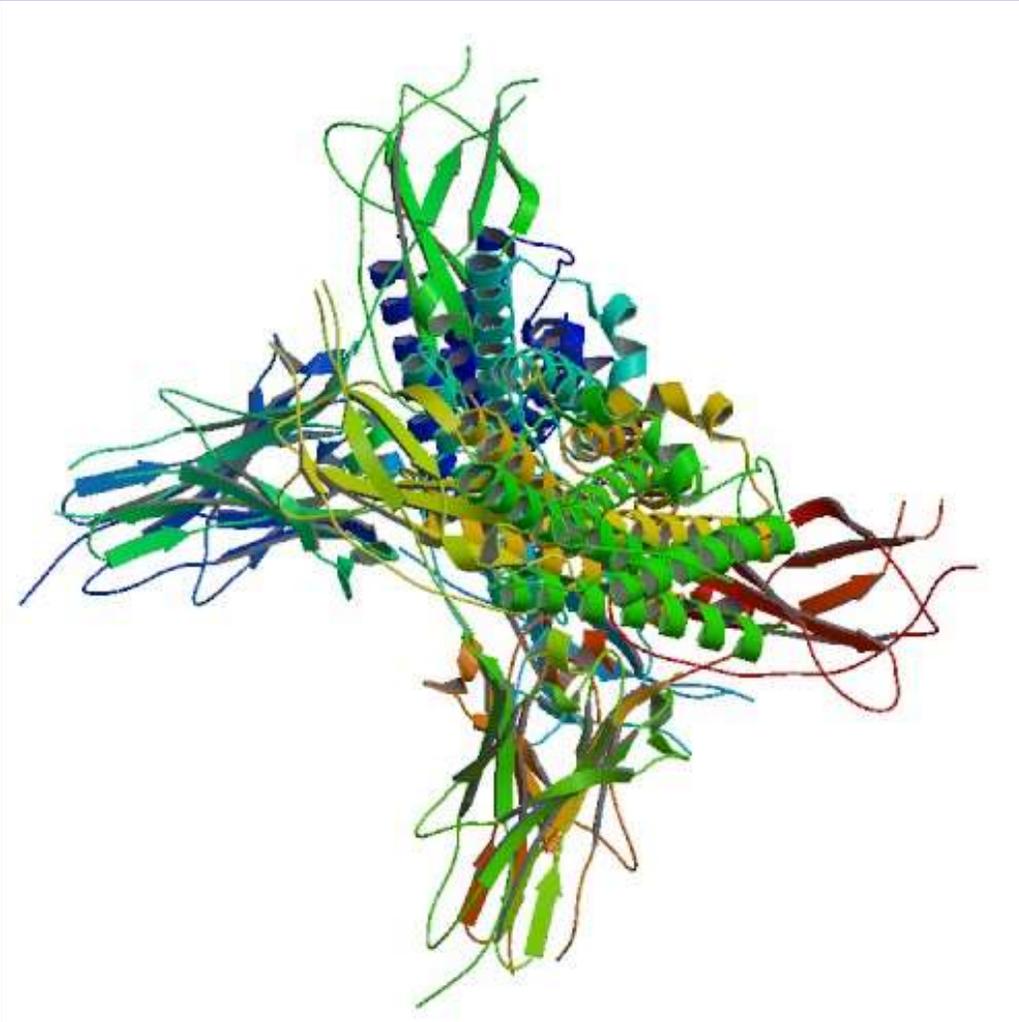


FILGRASTIM

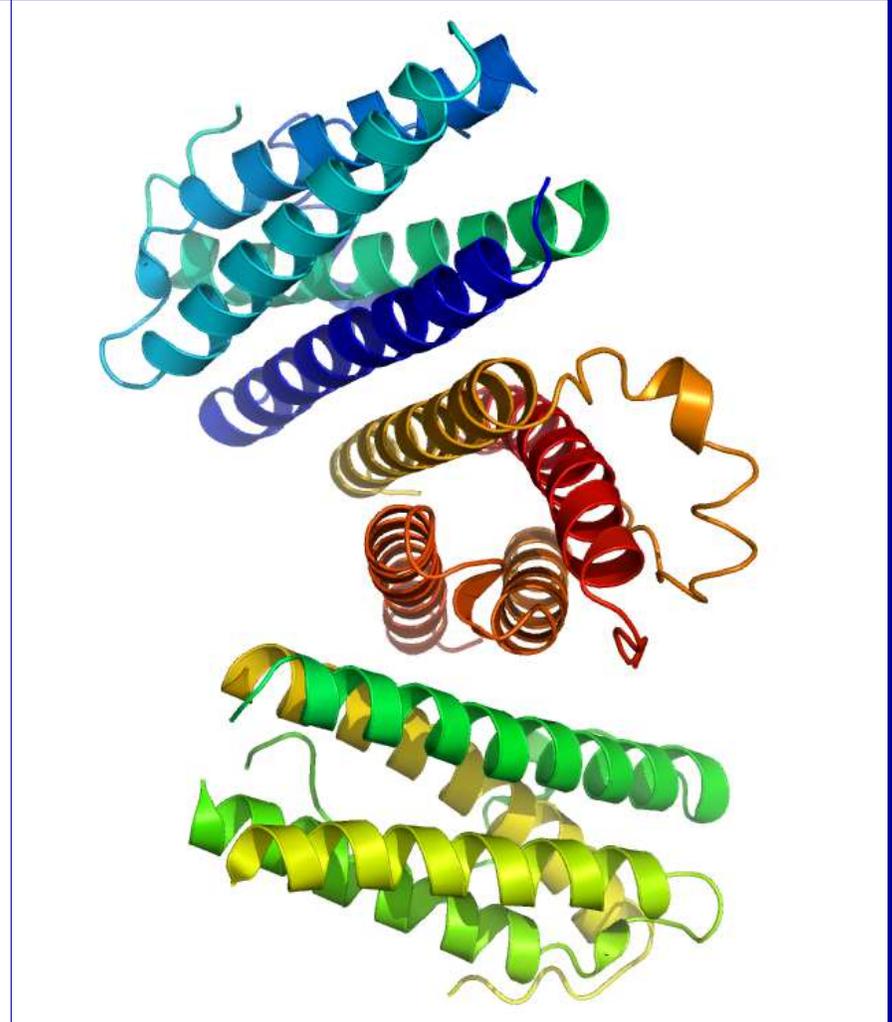
- Filgrastim is a recombinant human Granulocyte Colony-Stimulating Factor (G-CSF) with identical biological activity of endogenous human G-CSF, but differs since containing an N-terminal methionine residue and is not glycosylated



Filgrastim



G-CSF



FILGRASTIM

- It stimulates activation, proliferation and differentiation of neutrophil progenitor cells and it has been used in the treatment of patients with various neutropenic conditions
- It is known to mobilize hematopoietic stem cells (HSCs) from bone marrow into peripheral circulation, and for this reason, it is used to increase the number of hematopoietic stem cells in the blood before collection for HSCs transplantation



FILGRASTIM

- It exhibits significant neuroprotective effects in cerebral damage models, facilitating functional recovery in rats after stroke.
- It exhibit an anti-apoptotic effect through activating a variety of intracellular signaling pathways, including Janus protein tyrosine kinase/signal transducer and activator of transcription (JAK/STAT), extracellular-regulated kinase (ERK) and phosphatidylinositol 3-kinase/Akt (PI3K/Akt).



FILGRASTIM

- It is generally well tolerated, side effects are fever, cough, chest pain, joint pain, vomiting, and hair loss.
- More rare and severe side effects are splenic rupture and allergic reactions.
- The most frequent adverse reaction is mild to moderate medullary bone pain, reported by approximately 20% of patients, although this can generally be controlled using analgesics



G-CSF

- **Granulocyte-colony-stimulating factor (G-CSF)**, is a glycoprotein of 174–180 amino acids long and with a molecular weight of 19,600 Dalton: its gene is located on the long arm of chromosome 17, region 17q11.2-q12.8.
- It binds to a specific receptor, the G-CSF R or CD114, encoded by a gene on the short arm of chromosome 1 region 1p35–34.3, a protein 836 amino acids long and of 92,156 Dalton in molecular weight. GCSF-R is associated with signal transduction through the JAK-STAT3 pathways.



G-CSF AND PREGNANCY

- G-CSF and its receptor have been found on trophoblasts and in the decidua of several mammals, including human placenta.
- An anti-abortive role has been demonstrated for G-CSF in the animal models, and its depletion is indirectly involved in miscarriages.
- It has also been shown that G-CSF has a positive effect on trophoblast metabolism. G-CSF is secreted in follicular fluid and its levels correlated with oocyte competence and the implantation potential of corresponding embryos.



RECURRENT PREGNANCY LOSS (RPL)

- ESHRE Guidelines for RPL (November 2017) recommend the use of the term RPL to describe two or more pregnancy demises independently whether visualized in the uterine cavity.
- ESHRE Guidelines reserve the term “Recurrent Miscarriage” to describe cases where all pregnancy losses have been confirmed as intrauterine miscarriages.
- ESHRE Guidelines recommend that ectopic and molar pregnancies should not be included in the definition as well as implantation failure after IVF.



RECURRENT PREGNANCY LOSS

- Pregnancy loss is a common complication in early pregnancy, its prevalence ranges from 10 to 15.
- RPL is less prevalent, and it has been estimated that it affects approximately 1% to 2% of women, when defined as three consecutive pregnancy losses before 20 gestational week.
- Genetic abnormalities of the conceptus are recognized cause of RPL: the prevalence of chromosome abnormalities in sporadic miscarriage was 45%, whereas the prevalence of chromosome abnormalities in a subsequent miscarriage after preceding RPL was 39%. Array-CGH is the preferred technique to test chromosome abnormalities of conceptus.
- We perform the karyotyping of the pregnancy tissue of the miscarriage after or at the second losses before to define “unexplained” the case of RPL.



Filgrastim in RPL

- We started using Filgrastim in RPL in 1997, in a woman after five consecutive miscarriages who delivered a healthy baby.
- in 2009 we published a controlled trial on a group of patients fulfilling these inclusion criteria: age <39 years, more than four previous miscarriages, failure of previous treatments for RPL, and they had to be negative for all of the known causes of RPL, including normal karyotyping of embryonic tissues in the previous miscarriage.
- The study group underwent daily administration of recombinant Filgrastim 1 μ g (100,000 IU)/kg/day from the sixth day after ovulation until the occurrence of menstruation or to the end of 9 weeks of gestation.
- The control group consisted of 33 subjects who were treated with saline solution.
- The live births in women treated with Filgrastim were 82.8%, whereas in the controls they were 48.5% ($p = 0.0061$). None of the newborns showed any major or minor abnormalities.



Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: a randomised controlled trial

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BACKGROUND: Recurrent miscarriage (RM) is defined as the occurrence of three or more clinically detectable pregnancy losses in the first trimester. In most cases of RM, its aetiology remains unexplained. Granulocyte colony-stimulating factor (G-CSF), a cytokine, and its receptor are expressed in placental tissue. To investigate the effectiveness of G-CSF in preventing embryo demise, we administered G-CSF to women with RM.

METHODS: A randomised controlled trial in women with RM treated with G-CSF or placebo was conducted in one private reproductive medicine clinic. Sixty-eight women with unexplained primary RM, all with at least four consecutive miscarriages and negative for all clinical investigations, were selected. Patients were randomized for s.c. treatment with G-CSF ($n = 35$) ($1 \mu\text{g}/\text{kg}/\text{day}$) starting on the sixth day after ovulation, or with placebo ($n = 33$). Patients were randomized using a computer-generated randomization number sequence. Pregnancy outcome (delivery of a healthy baby without major or minor malformations) was the primary outcome measure.

RESULTS: In the group treated with G-CSF, 29 out of 35 (82.8%) women delivered a healthy baby, whereas in the placebo group, this figure was only 16 out of 33 (48.5%) ($P = 0.0061$, odds ratio = 5.1; 95% confidence interval 1.5–18.4). Significantly higher β -hCG levels were found in gestation weeks 5–9 in women treated with G-CSF versus placebo ($P < 0.001$).

CONCLUSIONS: Our data show that G-CSF may be effective in the treatment of unexplained RM. However, further studies are needed to confirm the effectiveness of this treatment in women with unexplained RM, refractory to conventional treatment.

The study was registered with a ICMJE recognized registry, the Clinical Trial.gov Protocol Registry System, with the number NCT00772122.

Table I Demographic data for women with unexplained recurrent miscarriage who were treated with G-CSF or placebo in the RCT

	G-CSF	Placebo
Number of patients	35	33
Age (years): when pregnancy started	34.9 ± 2.7	33.8 ± 2.9
BMI: when pregnancy started	27.4 ± 1.9	27.8 ± 1.8
Smokers (more than 10 cigarettes per day)	1	2
Number of previous miscarriages	5.5 ± 0.4	5.6 ± 0.3
Gestational week of miscarriage	6.1 ± 1.2	6.4 ± 1.1

Data are mean ± SD.

Table II Results of the study in patients treated with G-CSF and controls (placebo)

	G-CSF	Placebo	P-value
Number of live births (%)	29 (82.8)	16 (48.5)	0.0061
Number of miscarriages (%)	6 (17.2)	17 (51.5)	0.0061
Gestational week of miscarriage (mean ± SD)	6.0 ± 1.1	6.2 ± 1.0	0.6989
Newborn weight (g, mean ± SD)	3050 ± 220	3125 ± 240	0.3098
Side effects			
Skin rash	1	0	0.5147
Leukocytosis	2	0	0.2617
Pregnancy complication*	0	1	0.3535

*Pre-eclampsia.

Filgrastim in RPL

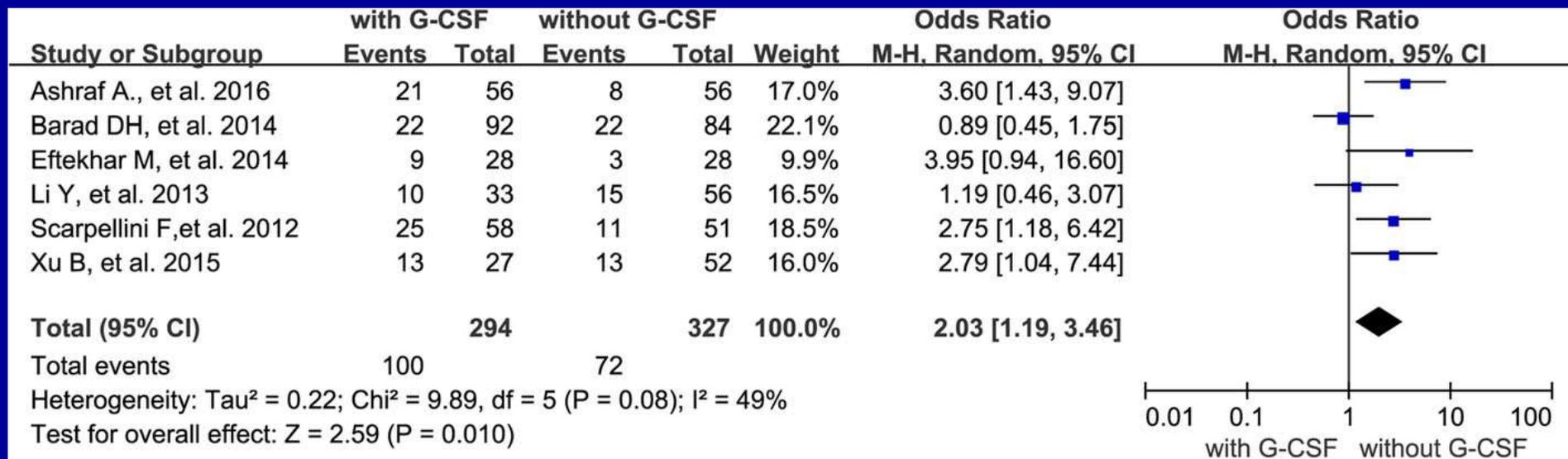
- To date we treated, in our clinical activity, with Filgrastim more than 500 women in 20 years experience, including patients with RPL and RIF.
- The livebirth rate in RPL was 78.8% out of 350 cases, really close to what reported for the study group in our original paper.
- In RIF patients we reported an overall livebirth rate of 52.2% out of 150 cases treated.
- We included in the study only women that performed negative to tests for known causes of RPL and show euploid karyotype in the previous miscarriage, and for RIF only women with a single euploid blastocyst transferred were treated with Filgrastim



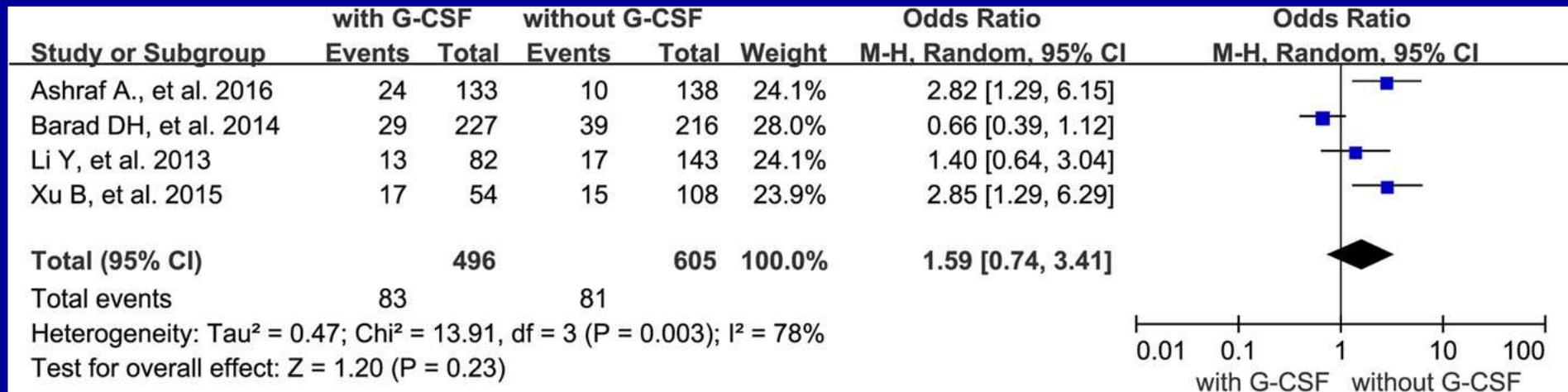
Filgrastim in RPL

- Wurfel et al in 2010 reported the effectiveness of Filgrastim in the treatment of women with Recurrent Implantation Failure (RIF)
- Several authors published reports about the treatment with Filgrastim in patients with unexplained RPL and RIF showing the usefulness of this treatment in improving the outcome of these reproductive disorders.
- Several reviews and meta-analysis have been published in the past two/three years showing the beneficial effects of Filgrastim treatment in unexplained RPL and RIF.
- However, some of these papers reported data obtained from non well selected groups of patients with RPL or RIF, and this may be considered a bias of these studies.

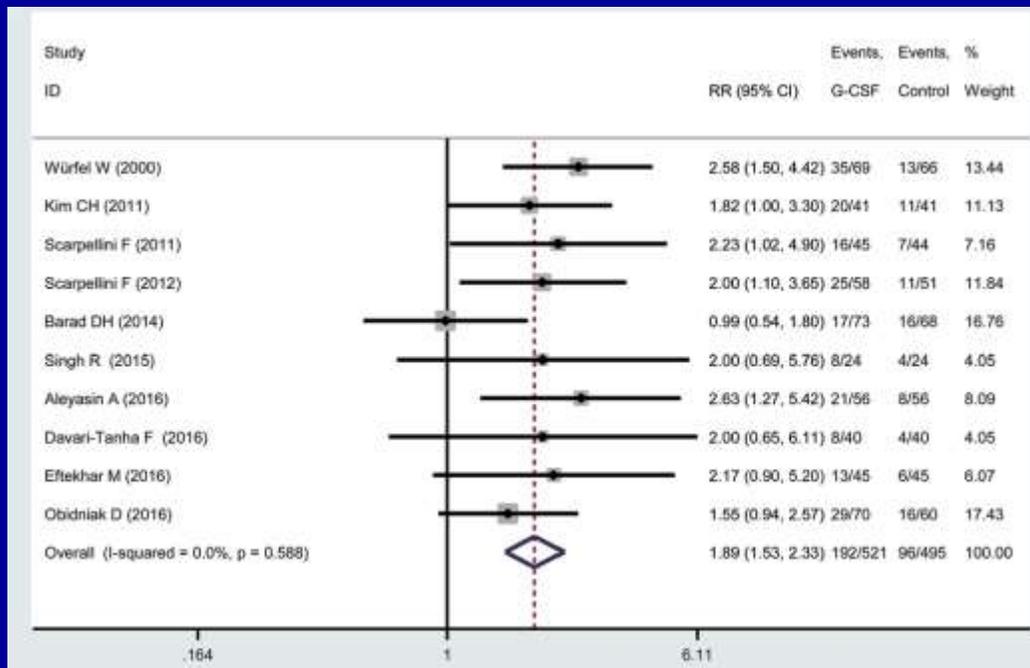




Forest plot showing the results of meta-analysis of studies evaluating the effect of G-CSF administration on pregnancy rate after ART

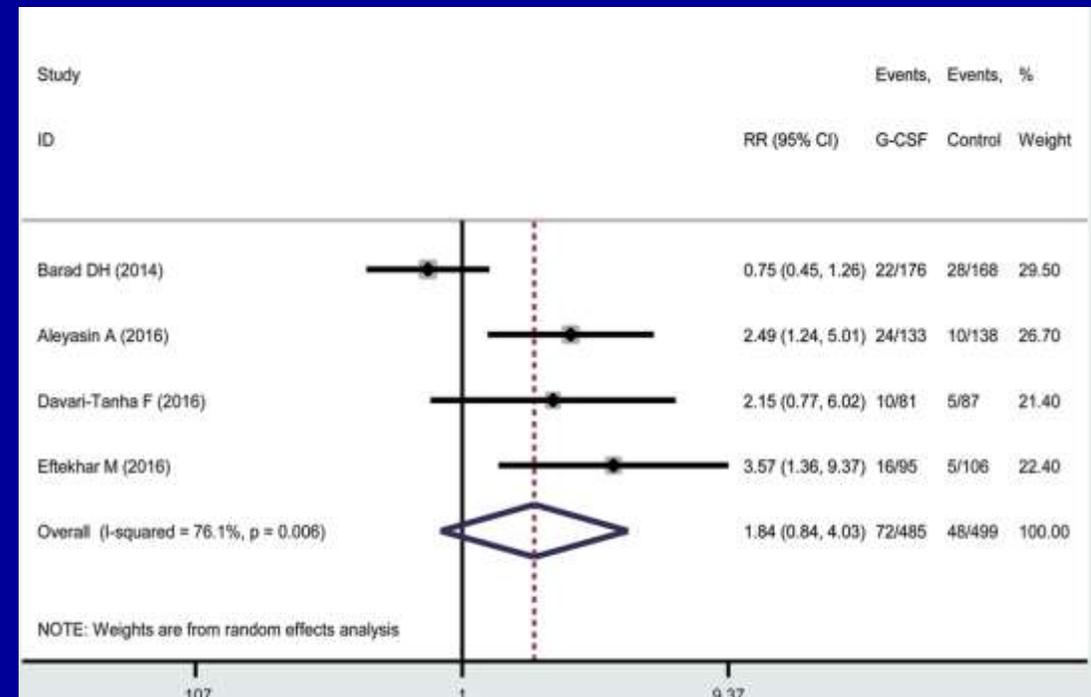


Forest plot showing the results of meta-analysis of studies evaluating the effect of G-CSF administration on embryo implantation rate after ART



Forest plot comparing the effect of G-CSF on CPR and IR in infertile women undergoing IVF/ICSI. The forest plot shows a benefit of G-CSF administration for CPR (a), but none for IR (b).

A random effect model was used for IR analysis because the included studies had substantial interstudy heterogeneity. Horizontal lines indicate 95% CIs; boxes show the study-specific weight; diamond represents combined effect size; dashed line indicates the overall estimate



Filgrastim Safety

- We did not observe any major adverse effect either in the women or in fetuses and in newborns on more than 500 patients treated with G-CSF in the implantation period and during the early pregnancy.
- Local skin rash was observed in the 3.6% of patients treated (resolved in few days)
- Fever in 2.6% of cases resolved in no more than three days
- Leukocyte count higher than 25 000/ml was observed in the 4.2% of cases (it was lowered by suspending the treatment for two or three days).



Filgrastim Safety

- Few data on possible toxicity in pregnancy of Filgrastim. Early experimental data on animal models showed placental embolism only in rabbits, with a dosage 1000 times higher than we used here, whereas in rats, mice and monkeys, no adverse effects were observed.
- Dale et al in 2003, in a series of 125 pregnant women treated over a long period with Filgrastim for chronic neutropenia, reported no adverse effects on pregnancy or the fetus.
- Pessach et al. in 2013 reported that in hematopoietic stem cell donation from healthy women donors during pregnancy and lactation treated with Filgrastim was safe in pregnancy.
- Boxer et al in 2015 reported no differences for pregnancy and neonatal complications in women treated with Filgrastim for chronic neutropenia during pregnancy compared to controls, also when treated during first trimester.
- Most of these data on pregnancy outcomes are obtained by patients treated during pregnancy with dosages of Filgrastim at least five times higher than we used in our patients.



Possible Mechanism of Action for G-CSF treatment in Recurrent Pregnancy Loss.

- There are only circumstantial evidences regarding the interaction of Filgrastim with trophoblast and immune system in RPL:
- G-CSF and its receptor are expressed in trophoblast cells throughout the pregnancy. The G-CSF-G-CSFR axis has been identified in the placental tissue as well as in decidua from early 1989 by Uzumaki et al,
- G-CSFR, expressed in trophoblastic cell lines, activated different signal transduction pathways, such as JAK/STAT, PI3K and MAPKs, which in turn increase Matrix Metalloproteinase-2 and VEGF secretion.
- Furthermore, G-CSF upregulates β 1 integrin and increases migration of human trophoblast cell line Swan 71,
- G-CSF in endometrial biopsies increases the expression of mRNAs for several genes involved in the implantation processes.

Possible Mechanism of Action for G-CSF treatment in Recurrent Pregnancy Loss.

- It is well documented the role of G-CSF in mobilizing mesenchymal stem cells from bone marrow to blood circulation, this characteristic is used to increase the stem cells concentration in the blood of donors in case of stem cell transplantation.
- G-CSF promotes Treg-cells mobilization.
- The mechanism involved for their mobilization seems to be the regulation of chemokine CXCL12 and its receptor CXCR4.
- Several authors described that the inhibition of CXCL12/CXCR4 axis is the key in the G-CSF-mediated bone marrow stem cells mobilization.
- The CXCL12/CXCR4 axis is also involved in Treg mobilization, since G-CSF decrease the expression of CXCL12 in these cells as well as the expression of its putative receptor expression, CXCR4.



Possible Mechanism of Action for G-CSF treatment in Recurrent Pregnancy Loss.

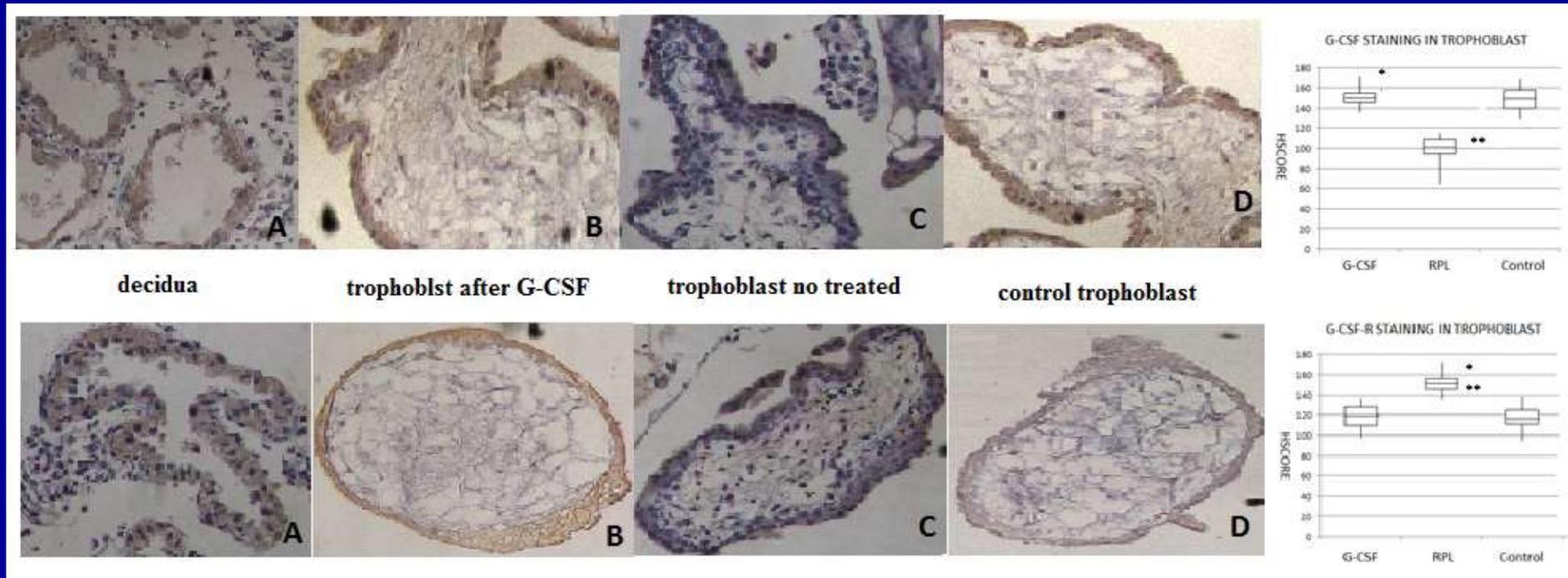
- Several studies have shown that G-CSF promotes the mobilization and proliferation of lymphocytes and dendritic cells, in particular Treg and DC2 cells.
- Our unpublished data show that women with RPL treated with Filgastrim had a remarkable increase of peripheral blood levels of Treg cells compared to normal pregnancy.
- In women with RPL treated with Filgastrim who subsequently miscarried again due to embryonic aneuploidy, there was an increase of Treg cells in the decidua compared to controls.
- In these cases we observed also an increase in of G-CSF and VEGF expression in the trophoblast compared to abortive and normal pregnancies
- a significant increase of β -hCG levels from the 5th through the 9th gestational week in Filgastrim treated pregnancies compared to control pregnancies was observed



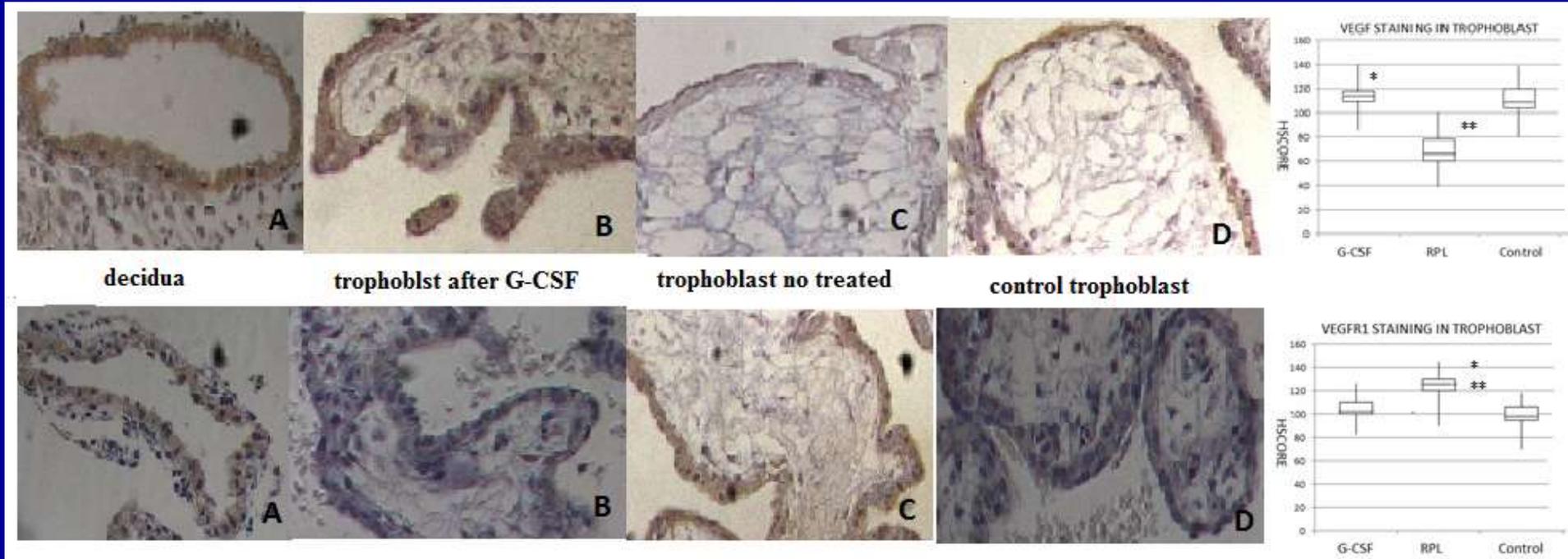
Treg in decidua of first trimester pregnancy with and without Filgrastim treatment



G-CSF and G-CSFR expression in trophoblast of first trimester pregnancy with and without Filgrastim treatment



VEGF and VEGFR-1 expression in trophoblast of first trimester pregnancy with and without Filgrastim treatment



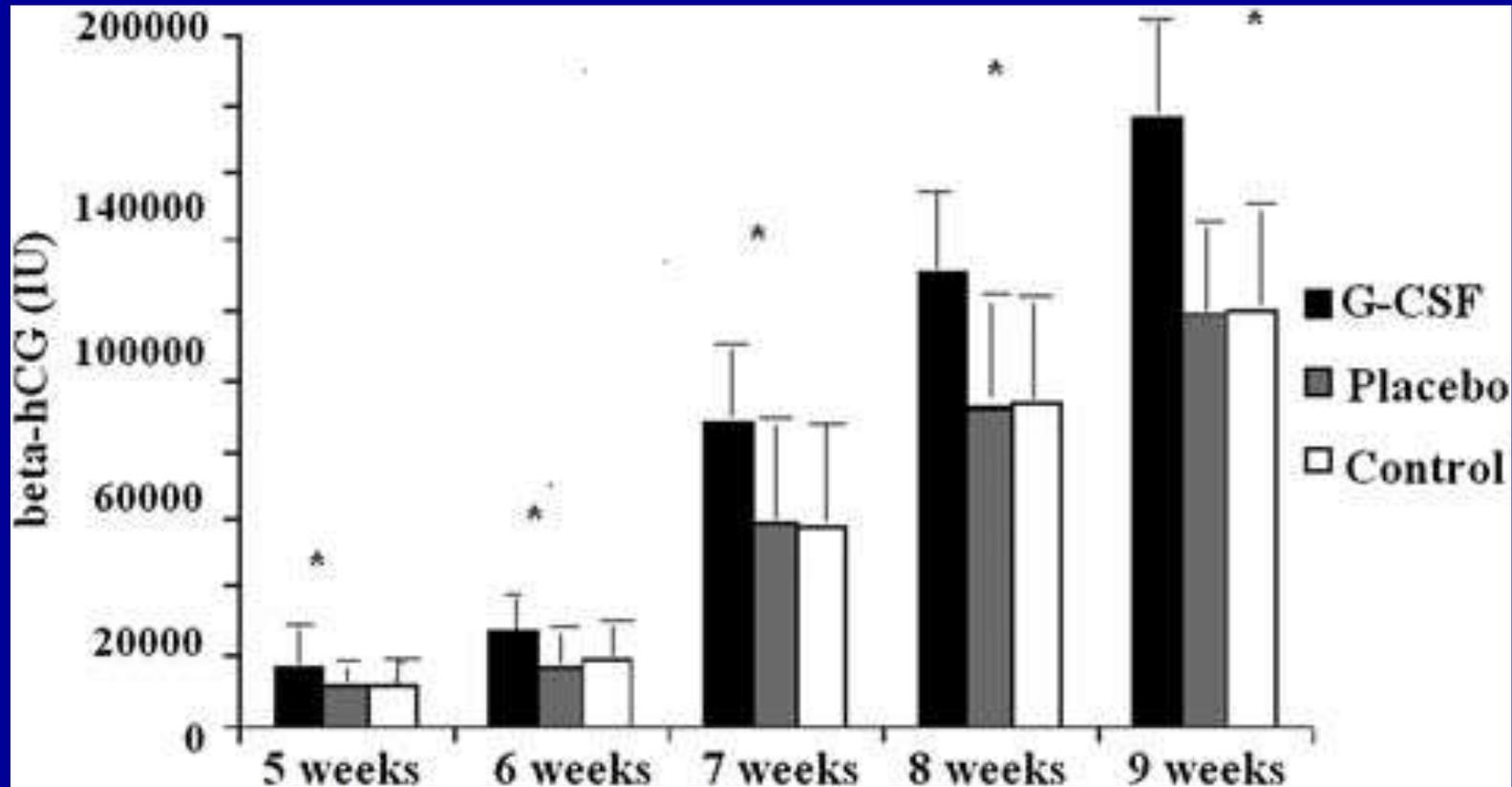


Figure 1 The data of b-hCG levels (mean \pm SD) were reported for each gestational week from the fifth to the ninth in the three groups: women with recurrent miscarriage (RM) treated with granulocyte colony-stimulating factor (G-CSF) (n = 29), women with RM treated with a placebo (n = 16) and normal pregnant women (n = 15). A statistical significant difference was observed (P < 0.001) in all weeks between the experimental group versus the placebo and normal pregnant women.

Conclusions

- Filgrastim, should be considered a safe and effective treatment for unexplained RPL cases
- Since aneuploidy is a recognized cause of pregnancy loss, and its frequency increases with female age, the genetic analysis of conceptus tissue with array-CGH should be mandatory before considering the Filgastrim treatment.
- The presence of an euploid embryo in the previous miscarriage is a mandatory condition for Filgastrim treatment in these women, considering also the cost of the treatment.
- For RIF the PGT-A should be considered before treating these patients with Filgastrim, in order to use this treatment only when a healthy blastocyst is transferred.
- Although there are effective treatments for RPL with clear immunologic origin, such as in antiphospholipid syndrome, in unexplained RPL there is no evidence for effective treatment. In these cases, Filgastrim should be considered first-line treatment, even though more studies are needed