

High pregnancy rates with administration of granulocyte colony-stimulating factor in ART-patients with repetitive implantation failure and lacking killer-cell immunoglobulin-like receptors

Sir,

As early as the year 2000, we reported on the use of granulocyte colony-stimulating factor (G-CSF) for IVF patients who failed to become pregnant after repeated embryo transfers (Würfel, 2000; Würfel, 2003). At that time, we administered 300 µg of Molgramostin in a single dose on the day of the embryo transfer. The pregnancy rate of patients treated with transfer on day 2 (average two embryos) was almost 43% compared with almost 20% in the placebo group.

The study by Scarpellini *et al.* (2009) now reports on continuous administration of G-CSF, evidently without any identifiable negative effects on the infants. Given this, we decided to run a pilot study to investigate whether continuous administration of G-CSF to patients with RIF (repetitive implantation failure) would be beneficial or not.

However, the initial criterion—patients' failure to conceive after repeated IVF or ICSI treatments—appeared to us to be too vague. Taking the publication by Hiby *et al.* (2008) as a basis we initially performed killer-cell immunoglobulin-like receptor (KIR) typing on patients with more than five unsuccessful IVF treatments or embryo transfers. Only patients who lacked the three activating receptors were accepted for the study; also included were patients suffering from long-term unexplained sterility (average 6.8 years) and lacking the three activating KIR genes. Groups overlapped to some extent as cases of unexplained sterility had often undergone (unsuccessful) IVF treatment.

The incidence of three lacking receptors (2 DS 1, 2 DS 3 and 3 DS 5) was very high in the group examined, at 78%. In addition, it was noted that patients lacked not only the three named activating receptors but also further receptors, so that in general the group was shown to lack five to seven receptors including the above-mentioned three activating receptors.

We performed IVF and ICSI treatment on the defined group with the target of a day 5 transfer. Patients received 13 million units of Granocyte™ (Lanogrostim) every 3 days in addition to the usual luteal support. In cases where insufficient numbers of fertilized oocytes were available, the transfer was performed on day 2 (particularly in cases of ovarian insufficiency).

Results for d + 5: Exclusively transfer of two blastocysts/morulae or compacted morulae

Number of patients	40
Number of treatment cycles	42
Clinical pregnancies (excluding biochemical)	31
of which abortions (clinical)	12
Pregnancy rate per treatment cycle/embryo transfer	73.8%
Abortion rate (clinical pregnancies)	38.7%

Results for transfer d + 2 (also including patients with significant ovarian insufficiency)

Number of patients	19
Number of treatment cycles	19
Clinical pregnancies of which abortion (clinical)	8 3
Pregnancy rate per embryo transfer	42%
Abortion rate	37.5%

The group we selected and defined certainly had a very poor prognosis. The pregnancy rates achieved were extraordinarily high both in the day 5 transfers and the day 2 transfers. However, the rate of clinical abortions is also high (biochemical pregnancies were ignored). In the meantime, we have also conducted a further pilot study in which G-CSF was administered to patients with a history of multiple unsuccessful IVF treatments or unexplained sterility, who had no KIR defects. The results were very poor, with pregnancy rates currently below 10% per embryo transfer.

We conclude from this that the use of G-CSF is an extremely promising additional method of treatment in cases where defects in materno-embryonic implantation communication can be shown. This applies in particular to KIR defects and, in this, particularly to the lack of the three activating receptors as described by Hiby *et al.* Where such defects were not present, results of G-CSF treatment were disappointing.

On the basis of the results of these pilot studies, we are currently planning a prospective randomized double-blind study under the initial criteria given above.

References

Hiby SE, Regan L, Lo W, Farell L, Carrington M, Moffat A. Association of maternal killer-cell immunoglobulin-like receptors and paternal HLA-C genotypes with recurrent miscarriage. *Hum Reprod* 2008;**23**:972–976.

Scarpellini F, Sbracia F. Use of colony-stimulating factor for the treatment of unexplained recurrent miscarriage: a randomised controlled trial. *Hum Reprod* 2009;**11**:2703–2708.

Würfel W. Approaches to a better implantation. *J Ass Reprod Genet* 2000;**17**:473.

Würfel W. Immuntherapie bei ART-Versagern. *Reproduktionsmedizin* 2003;**19**:108–109.

Wolfgang Würfel^{1*}, Claudia Santjohanser¹, Kaimo Hirv², Monika Bühl², Osama Meri¹, Ina Laubert¹, Irene von Hertwig¹, Klaus Fiedler¹, Jan Krüsmann¹ and Gottfried Krüsmann¹

¹Kinderwunsch Centrum München (KCM) (Fertility Center Munich), Lortzingstr.26, D-81241 München (Munich), Germany

²Zentrum für Humangenetik und Laboratoriumsmedizin (Center for Human Genetics and Laboratory Medicine), Lochhamer St. 29, D 82152 Martinsried, Germany

*Correspondence address. Tel: +49-89-244144-99/91;

Fax: +49-89-244144-41/42; E-mail: info@ivf-muenchen.de

doi:10.1093/humrep/deq106

Advanced Access publication on June 3, 2010

Reply: High pregnancy rates with administration of granulocyte colony-stimulating factor in ART patients with repetitive implantation failure and lacking killer-cell immunoglobulin-like receptors

Sir,

We read with very interest the letter of Prof. Würfel *et al.*, in which they reported the data of their pilot study in the use of G-CSF in ART patients with repetitive implantation failure and lacking killer-cell immunoglobulin-like receptors (KIR). They reported a high pregnancy rate and concluded that G-CSF is an extremely promising additional method of treatment in cases where defects in materno-embryonic implantation communication can be shown.

This study is different from ours, since we treated women with recurrent abortion and no patients with repetitive implantation failure. Our study evidenced that the G-CSF is a promising treatment in women with unexplained recurrent miscarriage: furthermore, our study showed that this substance may increase the trophoblast growth and metabolism since the elevated levels of beta-hCG observed in these women during treatment. It is really interesting to hear that also in ART patients the G-CSF may have a positive role in increasing implantation rate and embryo growth. We have a limited experience in the treatment of women with repetitive implantation failure, and no experience at all for patients with lacking KIR. However, in our few patients with repetitive implantation failure treated with G-CSF, we observed similar results to the ones reported by Dr Würfel *et al.* Even though they are very preliminary results, we encourage the authors of this pilot study to pursue in their study, since we think that G-CSF may have a relevant role in promoting cell growth in undifferentiated cells, such as it has been observed in stem cells and

may be a therapeutic tool in case of implantation failure. However, we want to underline that in case of implantation failure, the number of patients needed to reach statistical significance are very high, and consequently, multi-centre studies are warranted.

Fabio Scarpellini and Marco Sbracia*

Hungaria Center for Endocrinology and Reproductive Medicine (CERM), Rome, Italy

*Correspondence address. E-mail: marcandrea@hotmail.com

doi:10.1093/humrep/deq107

Advanced Access publication on June 2, 2010

The downstream effects of vitamin D in spermatozoa needs further study

Sir,

We are interested the article by Blomberg Jensen *et al.* (2010), where the expression of vitamin D metabolic enzymes in reproductive system of the male is described. In the discussion, the authors speculate that vitamin D regulates calcium ion concentration of spermatozoa. However, the following references do not support this speculation. The concentration of calcium ion in semen plasma or spermatozoa cytoplasm was not reported by Menegaz *et al.* (2009) or Uhland *et al.* (1992). To our knowledge, the vitamin D regulation of spermatozoa calcium ion channels or ionophores has not been reported, and the prolonged survival of spermatozoa in low concentration of vitamin D (Aquila *et al.*, 2008) may not be mediated by calcium ions. Although a positive effect of calcium ions on spermatogenesis was proven by Almeida *et al.* (2000), the effect of calcium on spermatozoa is complicated. The concentration of calcium ions in cytoplasm of mature spermatozoa is negatively associated with viability, but calcium inflow triggers the capacitation of spermatozoa (Hong *et al.*, 1984). The relative low concentration of calcium ions is maintained by calcium ATPase on sperm membrane. Vitamin D functions through several second messages (PKC, G-protein, cAMP) in different cells; however, the downstream of vitamin D in spermatozoa needs further study.

References

- Almeida SA, Teofilo JM, Anselmo Franci JA, Brentegani LG, Lamano-Carvalho TL. Antireproductive effect of the calcium channel blocker amlodipine in male rats. *Exp Toxicol Pathol* 2000;**52**:353–356.
- Aquila S, Guido C, Perrotta I, Tripepi S, Nastro A, Ando S. Human sperm anatomy: ultrastructural localization of 1 α ,25-dihydroxyvitamin D receptor and its possible role in the human male gamete. *J Anat* 2008;**213**:555–564.
- Blomberg Jensen M, Nielsen JE, Jorgensen A, Rajpert-De Meyts E, Kristensen DM, Jorgensen N, Skakkebaek NE, Juul A, Leffers H. Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract. *Hum Reprod* 2010;**25**:1303–1311.
- Hong CY, Chiang BN, Turner P. Calcium ion is the key regulator of human sperm function. *Lancet* 1984;**2**:1449–1451.