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

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of treatment cycles</td>
<td>42</td>
</tr>
<tr>
<td>Clinical pregnancies (excluding biochemical)</td>
<td>31</td>
</tr>
<tr>
<td>of which abortions (clinical)</td>
<td>12</td>
</tr>
<tr>
<td>Pregnancy rate per treatment cycle/embryo transfer</td>
<td>73.8%</td>
</tr>
<tr>
<td>Abortion rate (clinical pregnancies)</td>
<td>38.7%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of treatment cycles</td>
<td>19</td>
</tr>
<tr>
<td>Clinical pregnancies</td>
<td>8</td>
</tr>
<tr>
<td>of which abortion (clinical)</td>
<td>3</td>
</tr>
<tr>
<td>Pregnancy rate per embryo transfer</td>
<td>42%</td>
</tr>
<tr>
<td>Abortion rate</td>
<td>37.5%</td>
</tr>
</tbody>
</table>

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


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**Reply: High pregnancy rates with administration of granulocyte colony-stimulating factor in ART patients with repetitive implantation failure and lacking killer-cell immunglobulin-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 immunglobulin-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 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.

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**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 messengers (PKC, G-protein, cAMP) in different cells; however, the downstream of vitamin D in spermatozoa needs further study.

**References**


