

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.

Menegaz D, Rosso A, Royer C, Leite LD, Santos AR, Silva FR. Role of 1 α ,25(OH)₂ vitamin D₃ on alpha-[1-(14)C]MeAIB accumulation in immature rat testis. *Steroids* 2009;**74**:264–269.

Uhland AM, Kwiecinski GG, DeLuca HF. Normalization of serum calcium restores fertility in vitamin D-deficient male rats. *J Nutr* 1992; **122**:1338–1344.

Bo Yang*, Hang Sun and He Wang
Department of Urology, Xijing Hospital, Forth Military Medical University, Xi'an, China

*Correspondence address. E-mail: drboyung@yahoo.com.cn
doi:10.1093/humrep/deq114

Advanced Access publication on May 31, 2010

Reply: The downstream effects of vitamin D in spermatozoa needs further study

Sir,

Thank you for your interest in our study 'Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract' (Blomberg Jensen *et al.*, 2010). We agree with Dr Yang and colleagues that at the time of submission of our manuscript the effect of vitamin D on intracellular calcium in human spermatozoa had not been reported. Vitamin D has several

non-genomic actions, including a rapid increase in intracellular calcium in various cell types. Since spermatozoa are transcriptionally silent, and are affected by both calcium channel blockers and calcium, we speculated (supported by seven references) that vitamin D increases intracellular calcium in mature spermatozoa. Our suggestion has now been further supported by a recent study published by Aquila *et al.* (2009). Thus, it seems appropriate to speculate that vitamin D affects the calcium levels in spermatozoa.

References

Aquila S, Guido C, Middea E, Perrotta I, Bruno R, Pellegrino M, Ando S. Human male gamete endocrinology: 1 α , 25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) regulates different aspects of human sperm biology and metabolism. *Reprod Biol Endocrinol* 2009;**7**:140.

Blomberg Jensen M, Nielsen JE, Jørgensen A, Rajpert-De Meyts E, Kristensen DM, Jørgensen N, Skakkebaek NE, Juul A, Leffers H. Vitamin D receptor and vitamin D metabolising enzymes are expressed in the human male reproductive tract. *Hum Reprod* 2010; **25**:1303–1311.

Martin Blomberg Jensen*, Niels Jørgensen and Henrik Leffers
University Department of Growth and Reproduction GR, Rigshospitalet section 5064, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

*Correspondence address. E-mail: mabj@rh.regionh.dk
doi:10.1093/humrep/deq115

Advanced Access publication on June 2, 2010