Reply: G-CSF and repeated spontaneous abortions: what's new; comparability of inclusion and exclusion criteria in existing RCT-studies; deficiency is an indication, previous live births and 'unexplained' situations are not

Sir,

We thank Drs Santjohanser, Wagner, Hirv and Wurfel for their interest in RESPONSE study. We also thank Drs Sbracia, Scarepellini and Stamenov for their comments about our study.

The RESPONSE study investigated the efficacy of NT-100 in women with unexplained recurrent pregnancy losses (u-RPL). NT-100 (manufactured by its parent company—Nora Therapeutics) consists of an *E. coli*-derived human granulocyte colony-stimulating factor by recombinant DNA technology. Compared to the endogenous form of G-CSF, NT-100 included an N-terminal methionine residue and lacks O-glycosylation at Thr134.

We noticed that although the clinical evidence was limited, rhG-CSF was used widely as a treatment option for women with unexplained recurrent miscarriages through private IVF clinics and miscarriage clinics. This was based on retrospective observational studies and also on a small, single-center study (Scarpellini and Sbracia, 2009). RESPONSE study was designed to mimic the typical target population that a clinician may encounter in their day-to-day clinical practice in a UK-based NHS recurrent miscarriage clinic.

Drs Santjohanser, Wagner, Hirv and Wurfel made an observation on the possibility of inclusion of a sub-optimal patent population in our study.

The RESPONSE trial screened women prior to enrollment, with the key evidence-based RPL investigations. We did not exclude women based on secondary RPL as this population, in fact, represents a more severe phenotype than what is represented by ESHRE RPL definition of recurrent miscarriage, i.e. two or more miscarriages. It is noteworthy that several studies (Egerup *et al*., 2015, Christiansen *et al*., 2010) conclude that women with a history of recurrent miscarriage (RM) after a live birth (secondary RM) seemed most likely to obtain a potential beneficial effect of immune-based treatment options. We believe that women with secondary RPL are at increased risk of pregnancy losses and worthy of investigations and management. Therefore, we disagree with the observation regarding patient selection in the RESPONSE trial.

Dr Wurfel and colleagues identify a lack of dose determining studies for the use of rh-GCSF in RPL and, therefore, the potential bias of a sub-optimal dosing regimen in our study. Prior to conducting RESPONSE study, we completed a Phase 1, randomized, double-blind, placebo-controlled dose-escalation study in 48 healthy female volunteers. The dosing regimen for rhG-CSF in this Phase 1 study included woman with 65, 130, and 260 mcg daily. Vital signs and biochemical and immunology markers were performed. The changes in peripheral blood cell subsets were observed, which were consistent with a state of maternal–fetal immune tolerance. The changes included temporary induction of tolerogenic cell subsets and a decrease in pro-inflammatory cells. These changes were observed only in the multidose groups and not in the single-dose or placebo groups.

Drs Sbracia, Scarepellini and Stamenov identify a potential drawback regarding including women with uterine anomalies and gynecological conditions.

As stated in our original manuscript, we excluded women with congenital malformations and uncorrected major and minor intrauterine abnormalities. We also excluded women with a diagnosis of infertility due to any gynecological disorders. It is UK practice to investigate for endometritis, endometriosis and adenomyosis only in women who present with symptoms. Moreover, many of these conditions are not strongly associated with miscarriage, and even if women had these conditions, they could still have an immune problem that may have been amenable to rhG-CSF treatment.

We addressed the drawback of not performing fetal karyotyping in women who had miscarriages within the trial. However, performing routine karyotyping on pregnancy tissues is not cost-effective, and neither a UK practice nor a widespread practice anywhere in the world. More often, pregnancy tissues are not available for chromosomal analysis, and a woman can suffer a miscarriage of a chromosomally normal fetus, regardless of whether her previous losses were chromosomally abnormal or normal. Drs Sbracia, Scarepellini and Stamenov make subjective statements such as 'This appears to be mandatory' without providing any supporting evidence.

We agree that there is low-quality evidence based on human and animal studies (meta-analysis based on small, single-center studies) to suggest some benefit for the use of G-CSF to improve endometrial thickness, ovarian follicular function, oocyte quality and enhancement of embryo implantation. However, a systematic review of colony-stimulating factor supplementation (Siristatidis *et al*., 2013) in embryo culture medium in human IVF treatment concluded no meaningful improvement in clinical outcomes in all except one study. Furthermore, there is not enough evidence to prove the benefits of rhG-CSF in the peri-implantation period (unpublished study: THRIVE IVF study—ClinicalTrials.Gov identifier: NCT01864356).

We thank Drs Sbracia, Scarepellini and Stamenov for drawing the readers' attention to our high-quality trials in the NEJM (PROMISE trial, TABLET trial and PRISM trial). We note with concern that they are suggesting post-randomization exclusions, which would be a serious violation of the methodological principles of RCTs. We must emphasize the principles of intention to treat (ITT) and the importance of avoiding post-randomization exclusions.

Frustrating as it is to obtain negative outcomes associated with a RCT, our team is pleased that we answered a crucial, clinically relevant question.

Based on our observations from this large, multi-center, well-conducted, high-quality randomized control trial, we conclude that rhG-CSF does not improve pregnancy outcomes in women with unexplained RPL.

Conflict of interest

None.

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Can deep learning automatically predict fetal heart pregnancy with almost perfect accuracy?

Sir,

We read with enthusiasm the recent article by Tran *et al*. reporting the utilization of 'Deep learning as a predictive tool for fetal heart pregnancy . . . '. (Tran *et al*. 2019) The authors report the assembly and compilation of a large dataset consisting of time lapse video files of a total of 8836 embryos that were collected from eight clinics and labeled by fetal heart pregnancy (FHP) outcome. The reported area under the ROC curve (AUC) was astoundingly high, 0.93, indicative of a nearly perfect classifier. In fact, the ROC curve reports a perfect true positive rate 1 obtained with 0.72 specificity. The ability to predict FHP outcome with nearly perfect accuracy solely based on time-lapse sequences of embryo development lacking any information on endometrial receptivity means that the maternal factor in determining blastocyst implantation is negligible relative to embryo quality.

However, the claimed prediction of FHP as specified in the title is not supported by the training process. Only 1773 out of 8142 negative FHP-labeled embryos had actually been transferred to the uterus whereas the majority of these embryos (7063 embryos) had been discarded based on 'failed or abnormal fertilization, grossly abnormal morphology or aneuploidy from preimplantation genetic testing'. The clinical need is the evaluation of the implantation potential of embryos that are suitable for transfer rather than the discarded embryos that failed fertilization, showed abnormal fertilization or exhibited a grossly abnormal morphology. Moreover, FHP is not excluded by aneuploidy and determining negative FHP outcome with no direct evidence is not justified (Hassold and Hunt 2001).

To obtain further insight, we performed a simple exercise where a fictitious set of labeled embryos was considered that recapitulates the composition of the dataset used by Tran *et al*.: 8% positive FHP transferred blastocysts, 12% negative FHP transferred blastocysts and 80% non-transferred discarded embryos. Assuming that the video files of discarded embryos can be distinguished relatively easily from Day 5 transferred blastocysts, we considered accurate identification of all discarded embryos and only random prediction of FHP of the transferred embryos. Such a classifier is not predicting FHP—it distinguishes the dynamic appearance of discarded embryos from blastocysts. Under these assumptions, we also obtained AUC 0.93. With this result, we do not attempt to determine that the reported algorithm by Tran *et al*. generates random prediction of transferred embryos. However, concerns are raised as to which outcome is actually predicted and to what extent this prediction relates to embryo implantation potential. As long as the essence of the reported classifier remains unresolved, we alert the misleading title inadequately highlighting prediction of fetal heart pregnancy.

Conflict of interest

Y.K.T., A.B.M. and A.B. declare the ownership of a pending PCT patent application number PCT/IL2020/050120 protecting a machine-learning based assisted reproductive technology. A.B.M. is a medical consultant for Fairtility Ltd.

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