were counted before the samples were treated with erythrocyte lysis buffer to allow all CD45-positive leukocytes to be visualized. We omitted erythrocyte lysis buffer from the protocol used to prepare the second group of samples to optimize morphological preservation for the detailed analysis of leukocyte subtypes. In several cases, aspirates did not contain sufficient material for both sets of data to be readily obtained from the same sample. Despite this, sample numbers were clearly sufficient to satisfy rigorous statistical analysis; we therefore disagree with Dr Akkoyunlu’s assessment of our study results as less than convincing.

Dr Akkoyunlu suggests that specific antibodies could be used to identify leukocyte subtypes. In our study, we used standard morphological assessment of leukocyte subpopulations to accurately determine lymphocytes, monocytes/macrophages and polymorphonuclear leukocytes. The value of this approach is clearly illustrated in Figure 2 of our article, where the different leukocyte subsets are readily identifiable. Of course, we agree that the use of markers in addition to CD45 would allow further subtyping to be carried out. In this way, it would be possible, for instance, to determine the ratio of helper T to suppressor/ cytotoxic T cells. Similarly, NK cells could have been enumerated using, for example, CD16 and CD56. However, the use of CD163, as suggested by Dr Akkoyunlu, would only have reinforced our morphological determination of tissue macrophages/activated monocytes.

If we had been asking about the inflammatory nature of the process leading to the presence of leukocytes in the aspirates, then fine-tuned analysis would indeed have been appropriate. Such an approach might, for example, reveal whether the numbers/proportions of macrophages correlate with any other parameters of the aspirate. This type of analysis would ideally require the simultaneous determination of cytokines, an exercise of some magnitude, and beyond the scope of the study. Our article describes in some detail the confusing and sometimes contradictory nature of the literature concerning the cellular composition of human follicular aspirates. In the light of this, our purpose, as we explicitly stated, was simply to establish whether leukocytes found in follicular aspirates might reflect blood contamination, any further studies being warranted only once this basic question had been satisfactorily resolved. For this purpose, the assessments we used were more than adequate.

From a clinical perspective, any information we can glean on follicular heterogeneity has potential relevance to the vexing question of how we can optimize follicle selection during in vitro fertilization and embryo transfer. On the basis of our own observations as well as those described in other reports from the literature (e.g. Espey, 1980, 1994; Kawano et al., 2001), it seems perfectly reasonable to speculate that variations in the profile of leukocyte subtypes might relate to follicular maturity. Dr Akkoyunlu states that we should have included the measurement of different parameters that might reflect follicular maturation, such as hormone levels and follicular size. It would indeed be interesting if there are correlations between our own observations and other parameters. In that event, a link to follicular maturation would become a firm hypothesis rather than a mere speculation. Therefore, if Dr Akkoyunlu is suggesting that our observations themselves are weakened because they are not supported by including such data, then we cannot agree and reiterate that this was not the purpose of our study. If, on the contrary, it is being suggested that these studies are potentially valuable in future work, then we are happy to concur.

References

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Which is more counterproductive: a brief delay or ‘start anyway’?

Sir,

We read with interest the article entitled ‘Repeated testing of basal FSH levels has no predictive value for IVF outcome in women with elevated basal FSH’ by Abdalla and Thum (2006). Although we congratulate the authors on their work, we disagree with them that the study ‘...provides a specific answer to the question as to whether repeated testing of FSH for patients to bring their basal FSH levels down...would be beneficial for the patients’ treatment’ for the following reasons:

(i) The study refers to FSH levels that were generally measured in a menstrual cycle other than the actual cycle of IVF treatment. Given the well-documented phenomenon of intercycle FSH level variability (Brown et al., 1995; Buyalos et al., 1998; Juin et al., 2003), correlating IVF cycle parameters and outcome to an FSH level measured in a different cycle seems illogical and undermines the validity of the study results. Obtaining more than double the live birth rate in the high-FSH group somehow exposes the weakness of the study design (even though statistical significance was not reached because of the profoundly small number of patients involved).

(ii) Even in IVF cycles where the FSH and estradiol (E2) levels were measured within the same cycle of treatment, the
authors declared in the materials and methods section that ovarian stimulation in some of those cycles (actual proportion not provided) was carried out using the long luteal-phase down-regulation protocol. This meant that multifollicular stimulation was effectively started in the ovarian cycle subsequent to that when the FSH level was measured, further undermining the hypothesis behind group allocation.

(iii) The authors claim in the abstract conclusion that delaying treatment for women with high FSH could be counterproductive, as they may wait for several months, during which time ‘they are getting older and closer to the menopause’. Yet in the discussion section, the authors justified the study design by stating that the study was limited to a 12-month period ‘to negate the effect of age’, implying that ovarian ageing is unlikely to have a significant impact within a period of 12 months or less. To our knowledge, there is no evidence to show that delaying treatment by up to 3 months is detrimental to cycle outcome. By the same token, if the authors’ assumption was true, then briefly delaying treatment for women with high FSH could be counterproductive, especially when a short (flare-up) or antagonist stimulation protocol is used (Martin et al., 1996; Gurgan et al., 1997; Jurema et al., 2003). The authors also provided no explanation for the cause or significance of basal FSH inter-cycle variability.

(iv) The authors referred to the study of Bancsi et al. (2004), which did not use E2 measurement in conjunction with basal FSH levels, but did not include the studies showing the negative impact of high basal FSH level in a particular cycle on treatment outcome especially when a short (flare-up) or antagonist stimulation protocol is used (Martin et al., 1996; Gurgan et al., 1997; Jurema et al., 2003). The authors also provided no explanation for the cause or significance of basal FSH inter-cycle variability.

(v) Monitoring of basal (day 2) FSH and E2 levels with a view to starting ovarian stimulation using a short (flare-up) or antagonist protocol on day 3 of cycle in the presence of low hormone levels requires a 7-day-per-week clinical and laboratory services, which are not available in most UK IVF clinics, hence the unpopularity of this practice. In conclusion, only a randomized controlled trial could specifically answer the question as to whether repeated testing of basal FSH level is beneficial. Acknowledging the difficulty in undertaking such study and until it is completed, we believe that, in the presence of a high basal FSH, a pragmatic approach of offering three consecutive day 2 FSH and E2 measurements is appropriate. If the FSH and/or E2 levels remain high, the patient is extensively counselled and subsequent management is strongly dictated by her choice.

References


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Reply: Which is more counterproductive: a brief delay or ‘start anyway’?

Sir,

Thank you for inviting us to respond to El-Toukhy and Taranissi’s comments upon our paper (Abdalla and Thum, 2006). We provide a 7-day-a-week clinical service in our unit and could easily measure early follicular phase FSH levels for all patients. Indeed, as it is equally valid to measure the FSH on day 1, 2 or 3, any other unit offering a 5-day-a-week service could choose to do so. We suggest therefore that the reason the practice is unpopular is not related to the clinical service offered by units but rather to the lack of any clear evidence of benefit to patients from the practice.

The measurement of FSH is valuable for its prognostic value in identifying patients with a reduced ovarian reserve and possibly for assisting decision-making regarding the starting dose of gonadotrophins for stimulation. However, there is a wide variability of response to stimulation amongst patients with high FSH levels, and some patients with normal FSH levels will have an unpredicted poor response. We believe that the best test for ovarian response is to start a treatment cycle and stimulate the ovaries with FSH, having advised the patients during consultation of the potential for a reduced response. We will proceed with egg collection in such patients who have some follicular response, even if this is less than that expected in patients with a normal ovarian reserve.

The correspondents seem to suggest that using the long protocol is not appropriate for patients with high FSH levels (even if variable), although they provide many publications that suggest that the use of the flare or antagonist regime does not improve the response. Nevertheless, they later suggest that for patients with elevated FSH levels one should wait for a cycle in which the FSH is lower and use either the flare or the antagonist protocol. They provide no evidence why either of these protocols should be superior to or effective than the long protocol.

In our study design, we insisted that there should be no more than 1 year difference between the two treatment cycles so that the change in FSH or success rates could not be explained by a factor of time or ovarian ageing. The use of 12 months was a