Our unfractionated samples, the threshold for discriminating M540 bodies from human sperm suspensions so, for all of the results reported in our paper that involved sperm preparation by discontinuous gradient centrifugation, M540 body contamination would not have been an issue.

While it remains theoretically possible that the presence of M540 bodies affected our analysis of ’unfractionated’ semen samples, this does to seem to have been the case for our cohort of subjects. Thus, while we have recently been able to confirm the presence of M540 bodies in semen samples from our patients and donors, we found that these structures were present in insufficient numbers to influence our patient versus donor comparison.

This difference with Muratori’s analyses may be due to the fact that our patient population comprised unselected subjects attending our local IVF clinical who possessed semen quality that was essentially normal (See Fig. 1 of our original paper).

If, like Marchiani et al. (2007), we had selected patients with severe defects in their semen profile, including oligoasthenoteratozoospermia, then M540 bodies might well have had a more significant influence on our results. Further studies with more seriously compromised semen samples will be needed to establish this point.

In our unfractionated samples, the threshold for discriminating abnormally high levels of TUNEL and 8-hydroxy-2′-deoxyguanosine (8-OHdG) reactivity was around 40% for both criteria, in keeping with the fact that levels of TUNEL positivity and 8-OHdG formation were highly correlated (Aitken et al., 2010, Fig. 2). This correlation would not have been impacted by the presence of M540 bodies because the latter contain no detectable DNA and so should not have stained with an antibody against 8-OHdG. Again, further studies will be needed to establish this point.

Muratori et al. suggest that ‘The percentages of TUNEL in unselected semen are expected to be greater than those reported by Aitken et al., (2010)’. If M540 bodies stain positively with the LIVE/DEAD Fixable Dead Cell stain used in our study then clearly this would lower our reported percentage of TUNEL positive cells, as suggested. However, in reality, the TUNEL/PI method (which excludes M540 bodies) used by Muratori et al. (2008) gave a mean ± SD of 40.8 ± 16.3% TUNEL positivity in unselected patient samples, while our analysis of an equivalent group gave a virtually identical mean TUNEL value of 40.16 ± 16.2%. We therefore find little evidence that our data set was significantly impacted by the putative presence of M540 bodies.

Furthermore in our laboratory, microscopic assessment of TUNEL positivity by fluorescence microscopy, in order to focus attention exclusively on spermatozoa, generated results that were not significantly different from those secured by flow cytometry.

M540 bodies are interesting structures that deserve further attention to establish their origin and significance. However, we do not believe that they impacted upon the core conclusions of our paper which, we reiterate, were: (i) that sperm DNA damage is largely oxidative, (ii) that there is significantly more oxidative DNA damage and DNA fragmentation in the patient population compared with controls and (iii) that density gradient centrifugation significantly increases the levels of oxidative DNA damage seen in human spermatozoa.

**References**


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**DHEA administration in poor responders**

Sir,

We read with interest the randomized control trial by Wiser et al. (2010) published in Human Reproduction regarding the benefit of administering DHEA to poor responders.

Since the conclusion of this trial is that administration of DHEA is associated with a higher probability of live birth, we think it is probably useful to the reader to highlight some important aspects of this trial, which show that the data presented do not seem to support such a conclusion.

In the current trial, following randomization, patients were allowed to perform two IVF cycles. In this way, 17 and 16 patients in the DHEA and in the control group performed 26 and 25 cycles, respectively. Such a design makes it necessary to analyze the probability of pregnancy or live birth by using appropriate methods, which in this case is the cumulative probability of pregnancy or live birth as assessed by life table analysis or by Kaplan–Meier survival analysis.
A comparison of the probability of pregnancy can be performed by the use of the log rank test (Kaplan–Meier).

The authors, however, have used, according to the statistical methods described in their manuscript, the Fisher’s Exact test. It should be noted though that, this test requires that the observations are independent from each other, which, is not the case here, since some patients performed two cycles.

What is more important is that even by accepting the authors (inappropriate) choice of statistical methods (Fisher’s Exact test), the numbers they present in Table IV, do not support their claims of an improved probability of live birth, as shown by a P-level of 0.05. The application of a Fisher’s Exact test with the numbers provided in Table IV leads to a P-level of 0.099 and not 0.05 as the authors report.

Thus, even when analyzed by using inappropriate statistical methods, the data provided in this report cannot support the conclusion that administration of DHEA is associated with a higher probability of live birth.

Adopting DHEA as a beneficial intervention for the management of poor ovarian response should be guided by appropriately analyzed data originating from rigorously designed studies.

Reference


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Reply: DHEA administration in poor responders

Sir,

Kolibianakis et al. raised important questions in their letter regarding the methods of data analysis used in our study.

They suggested analyzing the data by Kaplan–Meier survival analysis. This analysis estimates the survival function from life-time data. It might be used to measure the fraction of patients living for a certain amount of time after treatment. In our study, the time between the first and second treatments was short (1 month) and was for only two trials. The women in the two study groups had no large differences between them that would justify calculating a survival analysis. However, for further and larger studies with longer exposure to DHEA, this analysis could be done, also.

On the basis of previous retrospective studies where DHEA has a beneficial effect, we assumed the same tendency in this prospective study, and felt it was reasonable to perform a one-tailed test. When we summarized both cycles of the two groups, we found a higher live birth rate among the DHEA group, 6 (23.1%) versus 1 (4.0%), respectively (P = 0.05). We erred in writing ‘two-tailed’ and not ‘one-tailed’ test in the Methods section.

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Dietary fat consumption and endometriosis risk

Sir,

We welcome the recently published data evaluating the relationship between dietary fat consumption and endometriosis (Missmer et al., 2010). However, we also feel some comments on the paper are appropriate.

First, we have some concern that the abstract may be misconstrued; readers may take the message that fish oil consumption might be beneficial in preventing endometriosis. The initial statement of the abstract indeed reads ‘Fish oil consumption has been associated with symptom improvement in studies of women with primary dysmenorrhea and decreased endometriosis risk in autotransplantation animal studies’. On careful examination of the paper we eventually understand that pain improvement was shown for primary dysmenorrhea only (Deutch, 1995) but without a link to endometriosis-associated pain. The second half of the sentence suggests a therapeutic effect in preventing endometriosis based upon animal data (Covens et al., 1988). The article only describes slightly smaller implants without any signs of apoptosis or cellular death, without evidence for prevention. We consider that the effect upon the transplanted endometrium is so limited that it might equally well be a consequence of a reduced inflammatory reaction masking the implant instead of evidence for regression.

The authors’ assertion that ‘These relations may indicate a modifiable risk’ is speculation and that ‘This evidence additionally provides another disease association that supports efforts to remove trans fat from hydrogenated oils from the food supply’ is a premature conclusion. Indeed, an association cannot prove a cause and effect relationship, and in this article the effect is so weak (with an OR of 1.26) that these may thus be spurious correlations. From the data and elaborate analysis, we would conclude that the effect of dietary fat upon the incidence or severity of endometriosis, if any, is marginal and unlikely to be clinically relevant. The data seem not to support the conclusion that fish oil consumption is beneficial for the prevention of endometriosis.

The diagnosis of endometriosis was made by laparoscopy in women with pain or infertility. Since the reported incidence of endometriosis in these women is over 70% (Koninckx et al., 1991), the association between the risk of undergoing a laparoscopy and fatty acid intake will therefore probably be as significant as the association between endometriosis and fatty acid intake. To us, this would rather suggest