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LETTER TO EDITOR

Antioxidant trials—the need to test for stress

Sir,

The paper recently published in Human Reproduction Open by Joseph et al (2020) is one of a series of clinical trials which have been published in the past 12 months that purport to show little impact of antioxidant therapy on pregnancy rates (Matorras *et al.*, 2020; Schistermanet *al.*, 2020; Steiner *et al.*, 2020). All of these trials have been well intentioned and carefully conducted however they all suffer from the same fundamental flaw which calls into question the validity of the conclusions reached (Aitken, 2020a,b).

The authors are absolutely correct in pointing out that oxidative stress is a significant cause of male infertility that allegedly contributes to the pathology observed in a 30–80% of all cases (Tremellen, 2008; Aitken and Baker, 2020). This range is very broad because we have very little consensus around the assays that should be used to detect oxidative stress in such patients. Should we be using lipid peroxidation assays such as the thiobarbituric acid assay? BODIPY C11? or flow cytometric detection of 4-hydroxynonenal? (Nakamura *et al.*,2002; Aitken *et al.*,2007; Moazamian *et al.*,2015). Alternatively, should we be deploying robust protocols for measuring oxidative DNA damage (Vorilhon *et al.*,2018) as a bioindicator of oxidative stress? At this stage, we are not only unsure of which assay to use but also which cells or fluids we should be monitoring—the whole unprocessed ejaculate? Isolated seminal plasma? The unfractionated sperm population? A subpopulation of selected spermatozoa that are likely to participate in fertilization? Blood? The result of all this rampant uncertainty is that clinicians are not screening their male patients for oxidative stress and are instead prescribing antioxidant therapy indiscriminately.

As pointed out previously (Aitken, 2020a,b), there is really no point in administering antioxidants to patients if oxidative stress is not making a significant contribution to their infertility. It is like giving insulin to everyone who comes into hospital in a coma—some will get better, some will die and, overall, any therapeutic benefit will be lost in the noise. It is therefore imperative that we reserve antioxidant therapy for those infertility patients who are actually exhibiting signs of oxidative stress. The situation is undeniably complex because such stress can come from many quarters including, age, smoking, obesity, infection, poor diet, the presence of a varicocele, exposure to environmental toxicants as well as electromagnetic radiation (Aitken and Drevet, 2020). As a result of this complexity, it is hard to predict exactly who is likely to be suffering from oxidative stress and so there is a desperate need for an appropriate diagnostic test that can be broadly applied.

The absence of such an agreed test is disappointing because in animal models there is definitive evidence that antioxidant therapy is extremely efficient in reversing infertility phenotypes generated by oxidative stress (Gharagozloo *et al.*,2016). The problem clinically is that we are racing to assess the value of antioxidant therapy before we have developed the necessary protocols to both detect oxidative stress within the infertile population and monitor its intensity in the face of antioxidant therapy. As a result, the trials undertaken are inconclusive and much valuable time and resource is wasted. All we shall ever learn from such studies is that the current clinical practice of giving antioxidants indiscriminately to subfertile males, will not generate significant benefits and we should therefore desist from such practice.

Thus, before any more antioxidant trials are conducted we should, as Joseph *et al.* (2020) acknowledge, develop, and validate an optimized test of oxidative stress that can be used to identify patients for whom antioxidant treatment is appropriate. We could then conduct appropriately controlled trials confident in the knowledge that the appropriate patient population is being selected. The antioxidant formulations we use should also be carefully considered and their efficacy demonstrated in animal models prior to clinical application, as we might expect as a matter of 'best practice' for any new therapeutic agent. Recognizing that oxidative stress might frequently be a contributory factor in a couple's infertility rather than the entire cause, I would also argue the oxidative stress marker used for patient selection purposes, should also be used as the primary endpoint in evaluating the therapy. All an antioxidant can ever do is decrease levels of oxidative stress. Whether this results in a pregnancy depends on many other male and female factors that are beyond the reach of even the efficacious of antioxidant formulations.

Conflict of interest

RJA is a consultant for Memphasys Ltd, a biotechnology company specializing in sperm isolation.

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