Methodological concerns regarding levels of vascular endothelial growth factor (VEGF) in serum of patients with endometriosis

Dear Sir,

We read with interest the article titled ‘Levels of vascular endothelial growth factor (VEGF) in serum of patients with endometriosis’ (Gagné et al., 2003). We would like to bring to your attention some methodological concerns that have arisen and may have caused significant bias in the conclusion.

First, concentrations of VEGF were measured on serum samples, but there has been no validation of serum VEGF as an indicator of the extracellular circulating VEGF levels at the time of sampling. VEGF that is stored in circulating platelets is released during clotting. Therefore serum VEGF concentrations may reflect blood platelet degranulation in vitro rather than VEGF synthesis by peripheral tissues (Verheul et al., 1997; Banks et al., 1998; Webb et al., 1998). In plasma, platelet degranulation is minimized by adding anticoagulants to the blood samples and, as a consequence, plasma VEGF concentrations are up to 20 times lower than the matched serum VEGF concentrations (Banks et al., 1998). Small differences in the circulating VEGF concentration between women with endometriosis and controls may have been masked by the measurement of VEGF released from the platelets. Citrated, EDTA-treated or heparinized plasma processed in glass tubes is the material of choice for measurement of circulating VEGF. However, when utilizing serum for VEGF measurement, it is advisable to correct the measurements to platelet count (Hormbrey et al., 2002).

Second, the conditions of processing are relevant and should be standardized. The authors state that the serum samples were kept at room temperature for a maximum length of time of 5 h before processing. Delay in the sample processing may have a deleterious effect on VEGF concentration. The serum VEGF concentration changes according to clotting duration (Webb et al., 1998; Hormbrey et al., 2002). Hormbrey et al. proved that there is no significant difference in VEGF levels in serum samples processed between 30 and 60 min after collection, but the difference becomes significant when samples are processed after 2–6 h (Hormbrey et al., 2002). In view of this, samples should be ideally processed after a standardized period of time (ranging between 30 and 60 min) and then immediately stored at –80°C until immunoassay. Moreover, even if a strict uniformity of clotting time could be applied to all samples, the interpersonal variation in generation of VEGF in clotted samples may make the interpretation of any observed difference between disease and control groups very difficult, and may invalidate the results (Banks et al., 1998).

In light of these considerations, the author’s conclusion that endometriosis ‘is not associated with a significant modulation in the levels of circulating VEGF’ cannot be justified on the basis of the presented data. We believe that meticulous processing of the samples collected from women with endometriosis could allow more meaningful results to be obtained.

References


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DOI: 10.1093/humrep/deh033