

THE ALPHA PAGE



SOLUBLE HLA-G (sHLA-G) A PREDICTOR OF IVF OUTCOME?

There is a worldwide thrust to limit the number of multiple births after IVF in an effort to reduce the associated morbidity and mortality of both mothers and offspring (reviewed in (1)). To achieve the goal of single births after IVF, it is necessary to identify which single IVF-created embryo has the greatest potential to give rise to a healthy baby so that one and only embryo can be transferred without decreasing pregnancy rates. At the present time a combination of morphological, biochemical, and genetic criteria is used to evaluate preimplantation embryo health. The ideal method to evaluate embryo health would be a completely noninvasive assessment of the embryo either through imaging of the unstained embryo or by analysis of an aliquot of the culture medium surrounding the embryo. Two recent studies have suggested that soluble HLA-G (sHLA-G) in the culture medium of IVF embryos can be used as a predictor of the chance of pregnancy success (2,3). In the first study, Fuzzi *et al.* (2) performed a prospective study in which embryos from 101 women (43 IVF and 58 ICSI; mean age for both groups = 36 years) were cultured in 285 wells containing 1–4 embryos per well. Detection of sHLA-G and transfer of up to 4 embryos were performed after 48 or 72 hs in culture. The results showed that of the sHLA-G positive wells ($n = 75$), 18 pregnancies resulted (24% pregnancy rate), whereas of the sHLA-G negative wells ($n = 18$), 0% pregnancies resulted (0% pregnancy rate). In the second study, Sher *et al.* (3) performed a retrospective study in which 201 women (201 ICSI; Group A ≤ 38 years and Group B 39–44 years) were cultured in 594 wells containing one embryo per well. Detection of sHLA-G and transfer of an average of 3.2 embryos were performed 46 h post-ICSI. The results showed that in both age groups the pregnancy rate was higher when the amount of detected sHLA-G was above the mean (sHLA-G “high”) compared to below the

mean (sHLA-G “low”). In Group A, for the sHLA-G “high” transfers ($n = 101$) a pregnancy rate of 71% was achieved compared to the sHLA-G “low” transfers ($n = 58$) where only a 22% pregnancy rate was achieved. In Group B, for the sHLA-G “high” transfers ($n = 29$) a pregnancy rate of 52% was achieved compared to the sHLA-G “low” transfers ($n = 13$) where only a 15% pregnancy rate was achieved. In both the Fuzzi and Sher studies detection of sHLA-G was performed by using a sandwich ELISA procedure with monoclonal antibody MEM9 as the capture antibody and monoclonal antibody W6/32 as the detection antibody. Although these results are tantalizing, we see several problems that need to be addressed before these results should be used for routine clinical application. First of all, both studies transferred multiple embryos, so the embryo or embryos that were the source of the sHLA-G could not be identified. Second, the monoclonal antibodies that were used were capable of detecting only one of the two known sHLA-G isoforms (HLA-G5 and not HLA-G6). Third, since both studies were performed on embryos that had not yet undergone zygotic genomic activation (ZGA), the source of the sHLA-G had to be of maternal rather than of embryonic origin. Fourth, at least one paper (van Lierop *et al.* (4)) has appeared that reports no detectable sHLA-G in any of the fresh or frozen supernatants from either 8-cell or blastocyst stage IVF embryos that were tested.

This last study tested only a small number of embryos and used different monoclonal antibodies from the Fuzzi and Sher studies, but their monoclonal antibody also could potentially detect only HLA-G5 and not HLA-G6. Thus, the positive results of the Fuzzi and Sher studies need to be reconciled with the negative results reported by the van Lierop group. It is clear that more research needs to be performed on the possible use of sHLA-G as a marker of healthy embryos. Ideally, testing for sHLA-G should be performed on culture medium from single embryo culture that is followed by single embryo transfer. However, the constraints of clinical practice probably preclude such a strict testing protocol. It has been suggested that research on the mouse functional homolog of HLA-G, Qa-2 protein, should be rigorously pursued (5). We have shown that Qa-2 (the *Ped* gene product) in the mouse influences pregnancy success (reviewed in 6,7). We suggest that it is desirable to assess the effect of sQa-2, the mouse functional homolog of sHLA-G, in carefully controlled experiments in a well-characterized mouse model system of preimplantation embryo development. In addition, a

nonhuman primate, the olive baboon, has been suggested as an excellent animal model for functional studies of HLA-G since Paan-AG has been identified as the likely functional homolog of HLA-G (8). Future results obtained from carefully controlled human studies, and animal model studies, should answer basic questions about the use of testing sHLA-G levels in embryo supernatants as a reliable marker for the prediction of pregnancy outcome after IVF.

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