False positive immunoreaction in products of conception

F G Barker, P Ozua, S Van Noorden

Abstract
False positive immunostaining for cytomegalovirus in products of conception was revealed using an avidin-biotinylated peroxidase complex. The cause was shown to be endogenous biotin. The use of a non-avidin-biotin method avoided the problem.

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Methods
Paraffin wax embedded sections (3 μm) from formalin fixed tissue were used. Endogenous peroxidase was blocked by incubating the tissue for 30 minutes in 0.3% (w/v) hydrogen peroxide in water. The primary antibody to CMV was a mouse monoclonal antibody (Dako, High Wycombe, UK; catalogue no. M757) diluted 1 in 100 in 0.01 M phosphate buffered saline (PBS), pH 7.2, containing 0.1% (w/v) bovine serum albumin and 0.1% (w/v) sodium azide. The antibody diluent was used instead of the primary antibody as a negative control. The tissue was then incubated overnight at 4°C. Binding was revealed by biotinylated goat antimouse immunoglobulin (Dako; catalogue no. E 433), diluted 1 in 500 in PBS, and streptavidin-biotinylated peroxidase complex (ABC); (Dako; catalogue no. K 377). Peroxidase was developed in PBS containing 0.03% (w/v) hydrogen peroxide and 0.025% (w/v) diaminobenzidine tetrahydrochloride dihydrate (Aldrich Chemical Company, Gillingham, UK; catalogue no. 26, 189-0). Nuclei were lightly counterstained with haematoxylin. In the light of the preliminary results further investigations were carried out, substituting a mouse peroxidase anti-peroxidase (PAP) complex (Dako; catalogue no. P 850), diluted 1 in 100 in PBS, for the ABC reagent.

Results
The nuclei of some of the endometrial cells were strongly positive (fig 2), but similar staining was found in the negative controls in which PBS had been substituted for the primary antibody (fig 3). We then demonstrated the same staining in the absence of primary antibody in five of seven cases of evacuated products from missed abortions retrieved from the surgical file. Application of the ABC

Figure 1  Superficial cells of secretory endometrium with enlarged nuclei from a case of missed abortion.

Figure 2  Immunoperoxidase stain using the ABC method for detecting CMV in the same case as fig 1.
the enzyme development solutions alone or substitution of a PAP complex for the streptavidin or avidin containing reagent did not produce nuclear staining, although control CMV containing sections showed typical positive staining with the PAP method.

Discussion
We believe that the nuclei of endometrial epithelial cells sometimes contain biotin during and shortly after gestation. Biotin may take the form of intranuclear inclusions, which increases the resemblance to viral infection. This topic has already been discussed in the literature but as it seems to be little known, we would like to emphasise the importance of including negative immunostaining controls. We advise that methods employing avidin be avoided when immunostaining gestational endometrium for CMV.

Figure 3 Negative control (PBS substituted for antibody to CMV) in the same case as fig 1.

Comparison of two automated quantitative immunoassays for the determination of C reactive protein concentrations

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Abstract
Two quantitative, automated methods for the determination of C reactive protein (CRP) were compared: turbidimetry (Cobas Fara II, Roche, Welwyn Garden City, UK) and fluorescence polarisation TDx, Abbott, Wokingham, UK). One hundred and twenty routine serum samples submitted for measurement of CRP were tested using both procedures. The results were compared using regression line analysis and showed a high degree of correlation ($r^2 = 0.99$, $X$ coefficient = 1.01, constant = 0.11). C reactive protein can be accurately measured using the automated turbidimetric method which can be recommended as an alternative to fluorescence polarisation.

Serum C reactive protein (CRP) concentrations rise and fall rapidly as part of the "acute phase" response. Despite the non-specific nature of this response, CRP measurement provides a sensitive indicator of inflammation in a variety of conditions and is an objective index of the clinical response. Early studies of the clinical use of CRP measurement were dogged by a lack of standardisation and of quantitative assays which could be performed quickly and conveniently. Recently, an international reference standard for proteins in human serum has been published. This, coupled with the availability of rapid, automated and quantitative assay systems, has facilitated the development of CRP measurement as a routine clinical laboratory investigation.

Fluorescence polarisation is currently in widespread use, while the alternative method,
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