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Improving placental block morphology using microwave-assisted fixation

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ABSTRACT
The histopathologic assessment of placenta is often marred by poorly fixed and processed sections. An audit conducted in the department resulted in 21% of placental blocks showing features of poor processing. A microwave-assisted fixation procedure was introduced using the Micromed KOS microwave oven (ABACUS-ALS, Brisbane, Australia) for 2 h at 45°C. This resulted in an improvement in the quality of the sections with a zero failure rate on a subsequent audit.

KEYWORDS
Histotechnology; microwave-assisted fixation; placenta

Introduction

The placenta is a highly vascular tissue often required for histopathologic assessment for confident clinical management. It is quite a dense organ engorged with blood, and for this reason, it often fixes poorly with slow penetration of fixative solutions. Thin 2 to 3-mm slices for routine tissue processing are usually difficult to obtain. This regularly resulted in poorly fixed and poorly processed tissue in paraffin blocks. A quality audit performed in our department resulted in the recognition of an unacceptable number of poorly fixed and therefore poorly processed placental paraffin blocks. Galvez and co-workers [1] have shown that microwave radiation under controlled conditions reduced neutral buffered formalin (NBF) fixation times without loss of specimen quality. A microwave-assisted fixation (MAF) procedure was instituted to alleviate the problem of poorly fixed tissue embedded in paraffin and a follow-up audit was undertaken after using MAF. This paper presents the results of these audits as well as the MAF procedure.

Materials and methods

Fresh placentas were received in the Histopathology department at the Children's Hospital at Westmead. After swabbing for microbiological analysis (if clinically warranted), the placentas were placed in 10% NBF and allowed to fix overnight (at least 18 h). Representative samples from the umbilical cord and placental body as well as a membrane roll were collected and placed in NBF for 4 h further fixation. The blocks were then processed using a Thermo-Shandon Excelsior™ Tissue Processor (ThermoFisher Scientific, North Ryde, Australia) using an overnight program through ethanol, xylene, and paraffin. Five micrometer sections were prepared and stained using a routine hematoxylin and eosin procedure (Rentsch Hematoxylin, Alcoholic Eosin Phloxine (RBH formulation) both from Australian Biostain, Traralgon, Australia).

An audit was designed to determine the extent of poorly fixed/processed placenta blocks using this processor. All 41 blocks processed on 1 day were assessed using the following criteria. After microtomy trimming, processed blocks exhibiting central areas of mushy tissue that appeared white after contact with iced water were considered poor. The morphology close to the poorly fixed and processed central area of the block was poor (Figure 3). Nuclei were smudged, lacking chromatin detail. Cytotrophoblast nuclei are not easily discernible and the chromatin details were lacking, including the normally distinct nucleoli. Hofbauer cells lack cytoplasmic vacuoles and granules that are usually evident in well fixed and processed placental tissue. The stroma has a homogenised 'parched-earth' appearance. Compare the section in Figure 3 to the section in Figure 4 which was taken of the better fixed and processed edge of the same block.

The MAF procedure involved microwaving placental samples inside processing cassettes in NBF for 2 h at 45°C using the Milestone KOS Microwave oven (ABACUS-ALS, Brisbane, Australia). A second audit assessing the same criteria as the first audit was performed after MAF on 40 placental blocks.

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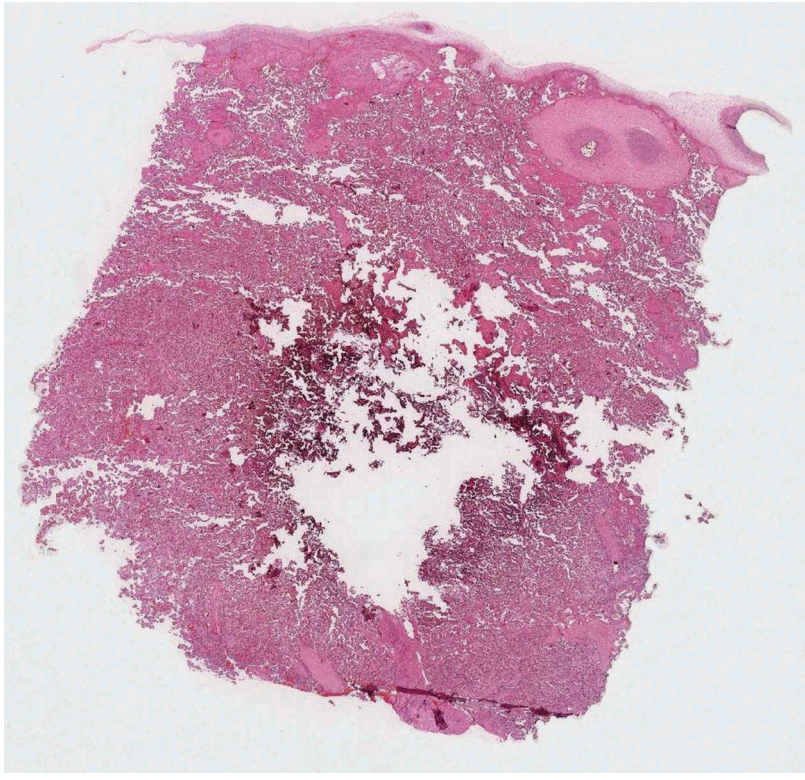

Figure 1. Section of placenta shows poorly fixed, poorly processed, central area of the sample (H&E, 1X).

Results

For the first audit, a total of 41 blocks were processed and of these 11 showed evidence of poor processing (27%). This poor processing exhibited itself in a trimmed block as central areas of white, mushy tissue after contact with iced water. Microscopically, the outer region of each stained section showed acceptable morphology whereas the central area failed to section (Figure 1, 2, 3 and 4). It was also noted that, at embedding, many of the processed tissue blocks showed dimples on the surface matching the holes of the processing cassettes (Figure 5). Following institution of the MAF procedure, the second audit was done on 40 blocks and none showed evidence of poor fixation or processing (using the criteria in methods). The

Figure 2. Paraffin block matching H&E section shown in Figure 1 shows the poorly fixed, poorly processed central area.

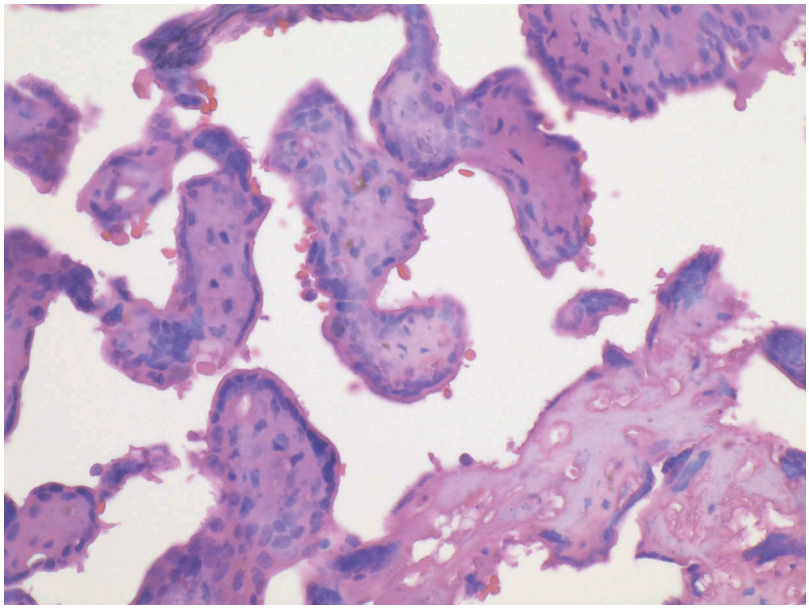

Figure 3. Morphology close to centre of the block. Note smudged nuclei, lack of cytoplasmic vacuoles and granules in Hofbauer cells, and homogenized 'parched-earth' stroma (see text). H&E, prior to using MAF, 40X.

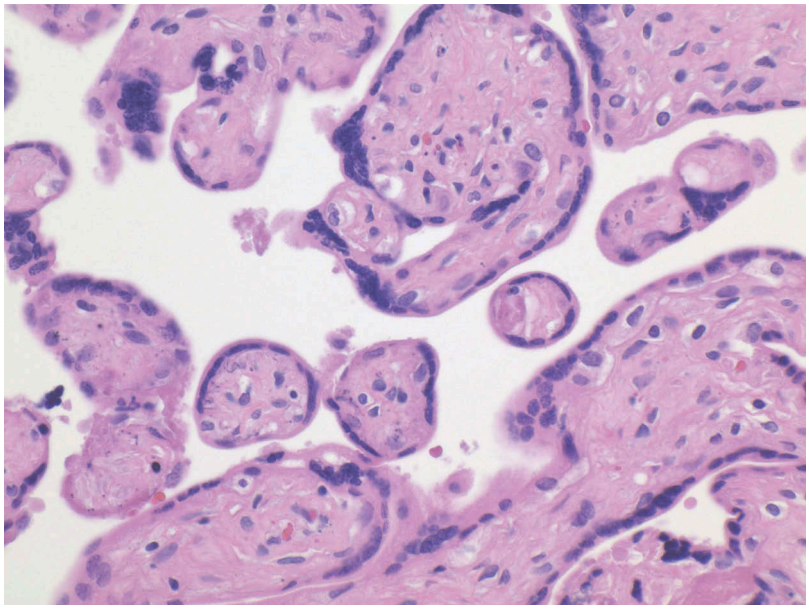

Figure 4. Same section as Figure 3, showing morphology closer to edge of block. Note better preserved nuclei, visible cytoplasmic details of Hofbauer cells, and distinct stroma H&E, prior to using MAF, 40X.

morphology was found to be quite acceptable in all blocks and slides assessed in the second MAF audit.

Discussion

Complete or at least adequate fixation with formalin is important in maintaining good microscopic morphology following tissue processing. The adversities against which a tissue must be protected commonly include passage through various processing solvents, embedding, sectioning, and staining. The quality of fixation is judged to be ideal when the finest details discernible in thin layers of living cells or tissues appear unchanged in the final preparations [2].

Formalin, like other fixatives, penetrates tissue slowly at a rate of about 4 mm in 24 h [3]. If tissue is not

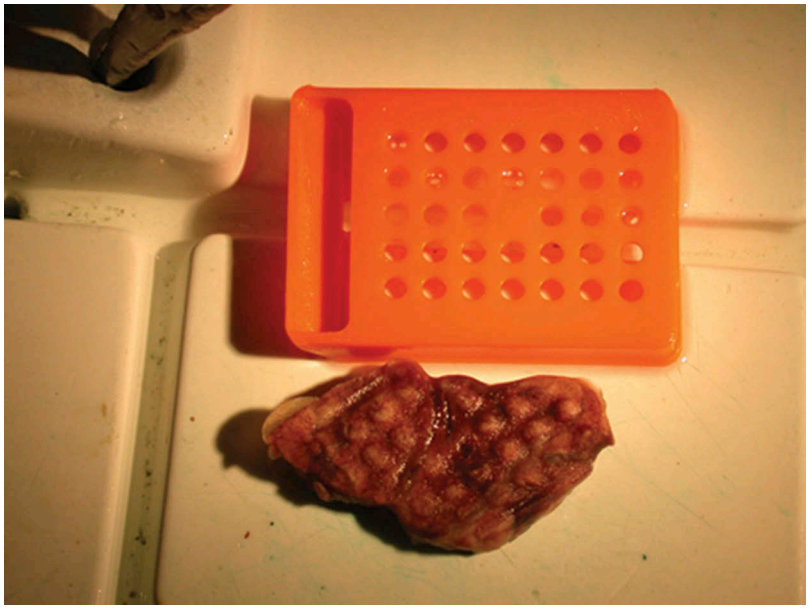

Figure 5. Processed placenta tissue with 'dimples' matching the fluid exchange holes of the processing cassette.

adequately fixed, then fixation continues in the alcohols used for processing. Alcohol causes shrinkage of cells as water is removed. This shrinkage appears to render it difficult to adequately clear tissue with xylene and impregnate with molten paraffin [4].

Chu and co-workers [4] recorded the morphological appearance of under-fixed tissue. In brief, 30 min of conventional formalin fixation resulted in unsatisfactory preservation of tissue, with cracking, and shrinkage, nuclear chromatin condensation, cellular distortion, and epithelial dissociation from basement membranes characteristic of under-fixed tissues. After 6–8 h of conventional formalin fixation, tissue showed mild swelling and rupture of cells, mild nuclear chromatin condensation, and mild cellular distortion.

MAF has been previously used by Abbuhl [5] to improve the fixation of fatty tissue specimens with the Milestone Mega TT Microwave oven (Milestone Inc.). The program used a 5-min ramp-up of temperature of the formalin to 60°C and this temperature was maintained with microwave stimulation for 55 min. Hasegawa et al. [6] on the other hand proposed that tissues always be microwaved in the fixatives and the temperature of the fixative after microwave irradiation remained below 40°C. To complete the cross-linking of the proteins, they recommended tissue specimens should be kept in the same fixative for at least 30–60 min after MAF.

The question still arises as to whether the improved morphology was caused by the microwave fixation, increased diffusion of formaldehyde fixative, increased microwave-assisted formalin fixation, or a combination of all three [7]. Microwave fixation uses heat to harden the tissue by a process of coagulation of proteins in the tissue. Irradiation in a microwave oven also generates internal heating in the central zone of the tissue causing an increase in the rate of fixative penetration. This phenomenon is called microwave stabilization and is defined as the fixation brought about exclusively by the irradiation effect of a microwave oven without using a chemical reagent [8]. Heat has been shown to increase the rate of formalin fixation [9]. Non-thermal effects have also been proposed including a possible increase in reaction rate by preferential orientation of the aldehyde molecules due to dipole rotation in the oscillating electric field. Other more speculative mechanisms such as direct interaction of the microwave radiation with the cell membrane have been suggested by Leonard and Shepardson [7]. They have shown non-thermal microwave fixation by cooling the specimen during irradiation produced poor fixation.

During the first audit, this study also noted that many poorly processed blocks showed dimples on the surface matching the holes in the plastic tissue cassettes. Extra care in ensuring the placenta samples is less than 3 mm in thickness before processing probably assisted in the improvement of the second audit.

MAF had a dramatic effect on fixation quality of placental blocks, improving from a 27% to a 0% failure rate. It is feasible to believe that MAF improved fixation occured by increasing the rate of formalin diffusion as well as the rate of formalin-tissue reaction.

Conclusion

MAF (2 h at 45°C) has been shown to improve processing of placental tissues resulting in improved morphology.

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Disclosure statement

No potential conflict of interest was reported by the author.

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