

# Evaluation of epigenetic marks in human embryos derived from IVF and ICSI

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in 2% PFA for 10 min after which the staining of DNA methylation was completed as described above using an AF488 secondary antibody. DNA was stained with 5 µg/ml 4',6-Diamidino-2-phenylindole for 10 min.

## Microscopy and image analysis

(1) Observations were made using an Olympus BX41 epifluorescence microscope. Images were recorded digitally with separate filter sets for YOYO-1<sup>TM</sup> and Alexa Fluor 594 using analySIS 3.1 (SIS GmbH). Greyscale images were pseudo-coloured and merged using Adobe Photoshop CS2. For three-colour images, the acquisition was performed with a Zeiss LSM 510 Meta confocal microscope equipped with a Plan-Apochromat 40x DIC oil-immersion objective. Serial optical sections were collected (minimum 7000 pixel size, z-step 0.46 µm). Selected images were assembled and pseudo-coloured using Adobe Photoshop CS2.

## Statistical analysis

Pearson's exact test was used for comparison (Microsoft Office Excel 2007). All P-values are two-tailed and 0.05 was considered significant.

## Results

IVF and ICSI-generated human embryos from the zygote through to the expanded blastocyst stage were fixed and prepared for DNA methylation evaluation using the 5MeC antibody. To visualise nuclear organisation and hence the status of chromatin, embryos were stained with YOYO<sup>TM</sup>, a sequence non-specific cyanine dye that quantitatively stains DNA.

In order to score embryos, a guideline for normal levels of DNA methylation and its organisation needed to be established. This guideline was informed by comparison to other mammalian systems and extensive experience in the mouse, where both active paternal and passive demethylation have been established (Mehk et al., 1987; Dean et al., 2001; Young and Beaujean, 2004). The consensus for normal DNA methylation and hence the epigenotype considered to be appropriate for samples in this study is presented in Fig. 1A. Human pre-implantation stage embryos appear to undergo limited passive DNA demethylation from the 2-cell up to the 8...16 cell stage or in the fifth cell cycle at which ~~de novo~~ DNA methylation appears to occur. Our observations are in close agreement with previously published results (Suika et al., 2004), and as such suggest they represent a good approximation of a normal DNA methylation profile.

Interestingly, fully expanded blastocysts (Fig. 1v) are asymmetric in terms of DNA methylation with a hypomethylated trophectoderm and a hypermethylated inner cell mass (ICM). This asymmetric pattern of DNA methylation is quantitatively different between species (Fig. 1B) but qualitatively conserved (Fig. 1C).



**Figure 1** Pre-implantation embryos staining guidelines. (a-v) Developmental profile for human pre-implantation staged embryos; (a-v) guideline for normalcy. Embryos were collected following either IVF or ICSI, fixed and stained with an antibody to 5-methyl-cytidine (5MeC) as a measure of DNA methylation and a quantitative DNA dye, YOYO<sup>1</sup>, to evaluate chromatin epigenotype. Embryos profiled in this figure represent the best estimate of normal pattern and intensity of DNA methylation of staged embryos from the 2-cell up to the expanded blastocyst. (b) Comparative illustration of qualitative features of DNA methylation asymmetry in the blastocyst. Examples of typical patterns for DNA methylation (red) and chromatin (green) in (a) bovine, (b) mouse and (c) human blastocysts. Collection of mouse and bovine embryos has been published previously (Santos et al, 2001; Santos et al, 2003).











