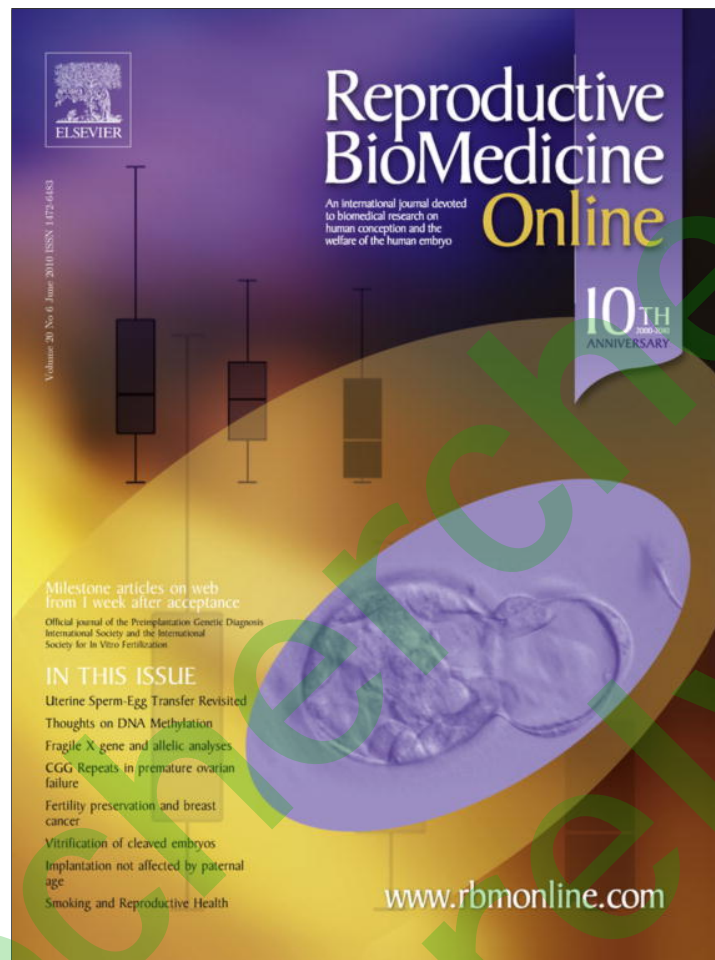


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## COMMENTARY


# DNA methylation and gene expression in IVF

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**Abstract** Recently, differences in DNA methylation patterns in placental and umbilical blood samples taken from children born after IVF and children conceived naturally have been reported. Since this may have an effect on gene expression, we highlight some of the biochemical/metabolic pathways in oocytes and embryos that might be relevant to methylation and imprinting during the process of human IVF. First, ovarian stimulation leads to elevated concentrations of follicular homocysteine which may have an effect on methylation. This should be compensated for by the systematic administration of folic acid and other B vitamins to patients. Second, there has been a trend to culture early human embryos in culture medium lacking essential amino acids. Consequently, methionine is not available during the first 3 days of in-vitro culture, a time when methylation is of major importance. We strongly recommend the use of culture medium with essential amino acids in human fertilization and early developmental stages. Finally, although all animals are, to some extent, a model for others, great caution should be exercised in extrapolating data; in particular, data from the mouse should not be assumed to be applicable to human embryology. 

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Katari et al. (2009) reported differences in DNA methylation patterns in placental and umbilical blood samples taken from children born after IVF and children conceived naturally. Although the numbers studied were limited (23 children), they found that some genes in IVF babies had lower methylation levels in placental tissue and higher methylation levels in umbilical cord blood tissue. They concluded that in-vitro conception is associated with quantitative differences in DNA methylation. Since this may have an effect on gene expression, we would like to highlight some of the biochemical/metabolic pathways in oocytes and embryos that might be relevant to methylation and imprinting during the process of human IVF.

Ovarian stimulation has been found to have an effect on homocysteine (Hcy) concentrations in follicular fluid; Sato et al. (2007) observed aberrant methylation patterns in oocytes retrieved after ovarian stimulation. Hcy concentrations are elevated in ovarian stimulation oocytes and Hcy is a well-known inhibitor of methylation. An elevated concentration of follicular Hcy after ovarian stimulation may

therefore have an effect on methylation and has a negative effect on IVF outcome (Berker et al., 2009; Pacchiarotti et al., 2007).

In the oocyte, Hcy can be recycled to methionine via the folate pathway, in a 'one shot' system that requires folic acid. *In vivo*, intake of folic acid decreases Hcy concentrations in follicular fluid. Contrary to what is observed in the mouse model, the cysteine beta synthase pathway is poorly expressed or absent in the human oocyte (Benkhalifa et al., 2010), with the result that there is only one pathway to remove Hcy and regenerate the methionine that is required for methylation. Methionine in the embryo's environment is thus of major importance, although high concentrations of folic acid can recycle Hcy to partly compensate for methionine deficiency.

It has been proposed, from experiments in the mouse, that essential amino acids might be toxic during early pre-implantation embryo development (Gardner et al., 1998; Lane and Gardner, 1998). This led to the practice of using media that lack 'essential' amino acids being used for

culture from fertilization to the 4- to 8-cell stage in human IVF. Consequently, methionine is not available during the first 3 days of in-vitro culture, a time when methylation is of major importance. Several authors have contested this 'essential amino acids' concept (Menezo, 2006; Summers and Biggers, 2003), pointing out that such culture medium is exactly the opposite of what is required by the embryo for correct imprinting. S-adenosyl methionine (SAM), synthesized by the embryo (Benkhalifa et al., 2010; Menezo et al., 1989), and group B vitamins are critical epigenetic regulators that have the capacity to affect DNA methylation and imprinting (Niemitz and Feinberg, 2004; Wolff et al., 1998; Xin et al., 2003) as well as histone H3 methylation (Dodge et al., 2004; Xin et al., 2003). Moreover, a deficiency of sulphur amino acids will also lead to increased sensitivity to reactive oxygen species damage, as well as to apoptosis, through decreased glutathione synthesis. It seems obvious that all amino acids should be available during fertilization and the zygote stage. We agree with Katari et al. (2009) that the design of IVF culture media warrants great care, especially in the absence of controlled metabolic experiments on human gametes and embryos.

Anomalies in histone methyl transferase activity will also affect gene silencing, which can lead to cytogenetic problems and altered DNA methylation, as observed in the Prader–Willi syndrome (PWS). It must be emphasized that CpG methylation in the PWS-1C site occurs after fertilization in humans, whereas in mice, PWS-1C methylation occurs during oogenesis (El-Maarri et al., 2001). Silent paternal alleles of *H19*, *Igf2*, *Grb10* and *Grb7* are aberrantly expressed and hypomethylated in simple medium but not in medium with amino acids in mouse embryos (Khosla et al., 2001). Micro-array technology has also demonstrated that expression of 114 genes is affected in culture medium without amino acids, compared with 29 genes with aberrant expression in culture medium with amino acids (Rinaudo and Schultz, 2004). Silencing of imprinted genes may constitute an epigenetic sign of cellular stress and tumorigenesis.

Aberrant methylation has been observed in DNA of spermatozoa from oligospermic patients (Kobayashi et al., 2007, 2009) and even the very early stages of preimplantation development require complete remodelling of methylation. This should not be overlooked when IVF is used for the treatment of couples with male factor infertility.

Finally, although all animals are to some extent a model for others, great caution should be exercised in extrapolating data; in particular, data from the mouse should not be assumed to be applicable to human embryology. Many commercially available culture media used routinely worldwide do not contain essential amino acids. Systematic administration of folic acid (Boxmeer et al., 2008) and other group B vitamins (B6, B9, B12) in patients undergoing assisted reproduction procedures is warranted, in order to minimize the risk of imprinting diseases.

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