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ARTICLE

H19 and *MEST* gene expression and histone modification in blastocysts cultured from vitrified and fresh two-cell mouse embryos

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Maryam Jahangiri received her BSc in biology from Iran at Guilan University in 2006. She continued her education at Royan institute in Tehran and began her graduate course under the supervision of Dr. Bahar Movaghar. Her graduate course was in field of cryobiology, and was entitled 'Effect of vitrification on histone modification of regulatory region of some imprinting genes in blastocysts resulted from two-cell mouse embryos'. She graduated in 2011 and currently works as a researcher in the Embryology Department at Royan Institute. Her major research interests are the molecular mechanisms involved in implantation. She also has undertaken research on epigenetic changes after vitrification.

Abstract Vitrification of embryos is a routine procedure in many IVF laboratories. The effect of vitrification on gene expression and some modifications of H3 histone in *H19* and *MEST* imprinted genes in blastocysts produced *in vitro* from non-vitrified and vitrified two-cell embryos was investigated. The expression level of the chosen imprinted genes increased significantly ($P < 0.05$) in experimental groups compared with in-vivo blastocysts (control group). H3K9me2 decreased, whereas H3K9ac increased in the experimental group compared with the control group. The increases in the expression levels of the imprinted genes, and the attendant changes in histone and chromatin status associated with in-vitro culture of embryos from the two-cell stage, are unaffected by prior vitrification and warming. In the present study, it was shown that such changes are solely caused by the effect of in-vitro culture, irrespective of vitrification. Although these genes are sensitive to environmental changes, vitrification seems to have no additional effect on these genes and on the histone marks, and can threfore be considered to be a process with minimum damage for embryo cryopreservation in assisted reproductive technology applications.

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KEYWORDS: blastocyst, *H19*, histone modification, *MEST*

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Introduction

Cryopreservation of embryos is an important component of assisted reproductive programmes. In some IVF centers, the number of embryos transferred into the uterus during the assisted reproductive procedure has been reduced to two or even one (Olivennes, 2000). In such cases, excess embryos are available for cryopreservation. Cryopreservation of surplus embryos increases the chances of conception, improves the cumulative pregnancy rates (AbdelHafez et al., 2010; Liebermann, 2009) and reduces cost of treatment (Wilding et al., 2010; Vajta and Nagy, 2006. Another benefit of cryopreservation is minimization of adverse effects of repeated ovarian stimulation (AbdelHafez et al., 2010; Shamonki and Oktay, 2005).

Among the different cryopreservation methods known, vitrification leads to higher survival rate of embryos (Wilding et al., 2010) because it prevents the formation of ice crystals inside and outside the cells (Rama Raju et al., 2005). The main disadvantage of this method is exposure of embryos to high concentrations of cryoprotectants in the vitrification solution, which may have a detrimental effect on them (Rezazadeh Valojerdi et al., 2009). The harmful effect of high concentrations of cryoprotectants can be minimized by reducing the exposure time of the embryos to the cryoprotectant to 30–40 s. Alternatively, using a combination of one or more cryoprotectants in the vitrification solution can also reduce toxicity effects on the embryos (Kattera and Chen, 2006; Rama Raju et al., 2005).

Preimplantation embryo culture is one of the strategies that can be used in assisted reproductive technology. Although it is a good method of selecting good-quality embryos, it can cause changes in gene expression and embryo metabolism. Recent studies have shown that children born through assisted reproductive technology show a high percentage of imprinting disorders, such as Angelman syndrome, Beckwith–Wiedeman syndrome and low weight birth (Ecker et al., 2004). Beckwith–Wiedeman syndrome is a congenital overgrowth syndrome in which multiple genetic and epigenetic mechanisms lead to alternation of expression in the 11p15.5 imprinted gene cluster (Lim et al., 2009).

Many genes involved in growth are epigenetically regulated. Imprinted genes *H19* and *MEST* play an important role in regulating fetal growth and development, placental function and postnatal behaviour (Liu et al., 2008). *H19*, which is located in an imprinting region on distal of chromosome 7 in mice (Doherty et al., 2000) and chromosome 11p15.5 in humans (Monk and Salpekar, 2001), expresses a non-coding RNA as a tumour suppressor (Gabory et al., 2010). This gene is expressed from maternally inherited allele (Doherty et al., 2000). *H19* is highly expressed during embryonic development in the endodermal and mesodermal tissues of the embryo. After birth, expression of *H19* stops or is drastically reduced in all tissues except in the skeletal muscle (Brunkow and Tilghman, 1991; Gabory et al., 2010). The expression pattern of *H19* in human fetal tissue is similar to that of the mouse (Lustig et al., 1994).

Mesoderm-specific transcript (*MEST)* is expressed in the paternal allele and encodes a alpha/beta hydrolase fold family enzyme with unknown function, which is located on proximal chromosome 6 in mice and chromosome 7q32 in humans (Huntriss et al., 2013). This gene is highly expressed in the early stage embryo, specifically in the mesodermal tissue (Kobayashi et al., 1997). *MEST* affects growth and early development of the embryo (Cattanach and Beechey, 1990; Kobayashi et al., 1997), and its disruption causes embryonic growth retardation (Lefebvre et al., 1998). In mice, loss of imprinting of *MEST* is associated with altered growth (Shi et al., 2004). Some studies have shown that assisted reproductive technology influences DNA methylation and expression of *MEST* in humans and mice. It has been reported that hyper methylation of the *MEST* gene in differentially methylated region leads to Silver–Russel syndrome, which is thought to be caused by IVF (Huntriss et al., 2013; Imamura et al., 2005).

Imprinting is the result of epigenetic change that begins in gametogenesis and is sustained with transfer to zygote and through cleavage in embryo. Imprinted genes are generally located within imprinted domains in which a region of differential DNA methylation is required to establish monoallelic gene expression (Pedone et al., 1999). Some studies have shown that ART techniques can change gene expression, and these changes are not caused by changes in DNA sequences but by epigenetic factors (De Rycke et al., 2002). Common mechanisms that mediate epigenetic changes include DNA methylation and histone modifications (Morgan et al., 2005).

The importance of histones in chromatin structure and transcriptional regulation through methylation, acetylation or phosphorylation of the amino termini is well known. These histone modifications can be either repressive or permissive for transcription, depending on their location and context (Kouzarides, 2007). It has been established by investigators that acetylation of lysine 9 of histone H3 (H3K9ac) or H3 trimethylation on lysine 4 (H3K4me3) are highly associated with active chromatin, whereas H3K9me and H3K27me3 are correlated to transcription repression (Lee et al., 2006).

Li et al. (2005) analysed H3K9me, H3K4me and H3K4ac in regulatory region of *H19* in CTCF site III of imprinting control region (ICR), which are located 2–4 kb upstream of the *H19* transcription site (Szabo et al., 2004) in embryonic stem cells derived from IVF blastocysts. Their results showed an increase in lysine 4 methylation on the paternal chromatin and a gain in lysine 9 methylation on the maternal allele. They suggested that IVF and culture media might cause DNA methylation errors and histone modifications in *Igf2*/*H19* genes (Li et al., 2005). CTCF is a member of BORIS plus CTCF family, and encodes a transcription factor that binds to the DNA sequence at specific sites (Cuddapah et al., 2009) and acts as an enhancer blocker and barrier. It prevents interaction between promoter, enhancers and silencers and interferes in transcriptional activation and repression. CTCF regulates interplay among DNA methylation, chromosome structure and gene expression (Phillips and Corces, 2009).

Epigenetic modifications of DNA and chromatin are important for genome function during development. Epigenetic modifications have an integral role in the regulation of fetal growth and development. As a result, any epigenetic deregulation can result in fetal growth abnormalities (Piedrahita, 2011). In this study, the effects of vitrification and embryo culture on expression of some important genes in embryo development were evaluated. The relationship between expression of these genes and modifications of a few histone marks (H3K9me2, H3K4me3 and H3K9ac) in their regulatory regions were also studied.

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