## A remarkable finding for embryologists to choose the best protocol in IVF procedures

<u>Asma. Momeni</u><sup>1</sup>; Tahereh. Haghpanah<sup>1</sup>; Seyed Noureddin. Nematollahi-Mahani<sup>1</sup>; Sareh. Ashourzadeh<sup>2</sup>; Seyed Hassan. Eftekhar-Vaghefi<sup>1.3</sup>

1. Anatomical Sciences Department, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.

2. Afzalipour Clinical Center for Infertility, Kerman University of Medical Sciences, Afzalipour Hospital, Kerman, Iran.

3. Kerman, Department of Anatomy, Kerman Branch, Islamic Azad University, Kerman, Iran.

Since the developmental stage of oocyte is a challenging issue in the success of vitrification, this study investigated the effects of vitrification, before and after in vitro maturation, on the survival and maturation rates, developmental competence and the expression levels of genes involved in apoptosis, oxidative stress and epigenetic modifications. Mouse germinal vesicle (GV) oocytes were divided into four groups: fresh in vitro matured oocytes without vitrification (fIVM), in vitro matured oocytes after vitrification (vIVM), in vitro matured oocytes before vitrification (IVMv). In addition, in vivo matured oocytes (MII) were used as control. After oocytes collection, maturation and survival rates as well as the intracellular reactive oxygen species (ROS) level were evaluated. Also, the expression level of various genes was analyzed by qRT-PCR. In addition, following artificial activation (parthenogenesis), the developmental competence of oocytes to the blastocyst stage was evaluated. A significant decrease in maturation rate and survival of vIVM oocytes was observed compared to fIVM and IVMv oocytes. Intracellular ROS levels were significantly increased in both vitrified groups compared to the fIVM group, and no significant difference between vitrified groups. proapoptotic genes; BAX and Bcl2 as well as genes related to oxidative stress response Hsp1a, Hsp1b and SOD1were significantly increased in the vIVM group compared to the IVMv group. Interestingly, epigenetic regulators genes DNMT1, DNMT3a and DNMT3b were highly

expressed in IVMv oocytes along with a decrease in the artificial activation rate compared to the vIVM oocytes. Our results indicated that despite observing more negative effects of vitrification before IVM on the survival rate and maturation as well as apoptosis status, less epigenetic changes in vIVM oocytes can make this process a better option in the treatment of infertility than IVM of oocytes followed by vitrification, a hypothesis that needs to be investigation in human oocytes.