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COMMENTARY

Lack of reproducibility in oocyte vitrification calls for a simpler (whether semi-manual or automatic) and standardized methodology

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The article of Tannus et al. 2018 [1] in this issue of the journal brings to the forefront an important issue in oocyte vitrification: the lack of reproducible results. The case reported is important because it shows that even in the same IVF lab and with the same methodology carried out by embryologists (senior or young) of the same team, the results differ dramatically. Time has arrived to standardize the methodology since, as noted by previous colleagues [2], there are too many protocols for vitrification and even subtle changes may ultimately have a profound impact on the outcome.

Vitrification differs from the slow-freezing technology and it is helpful to clarify the differences and the terminology. The process of vitrification is the solidification of liquid material into a glass-like form without the formation of ice crystals. Its introduction and widespread adoption resolved two of the main reasons for oocyte damage during slow freezing: membrane chilling injury and the lethal effect of ice crystal formation. The chilling injury of the oocytes is avoided by the fast cooling and warming rates of the process which basically outruns the time needed for membrane phase transitions to occur. Ice crystals are avoided due to the high viscosity of cryoprotectants in the solution and by keeping the biological samples in a small volume (minimal drop size (MDS)) and a high cooling rate [3]. The terms cooling and warming shall be used for vitrification, while freezing and thawing are related to ice crystal formation and are appropriate for slow freezing.

Many factors determine the success of oocyte vitrification; among them are the following: the composition of the equilibration solution (type and concentration of cryoprotectants (CPs)), the method of exposure (how many steps, time, and temperature of exposure), and the type of carriers utilized (open or closed system, surface, or tubes). At present, there are many carriers on the market and some of them do not allow reaching of high cooling or warming rates (which should be ~ 20,000 °C/min). The size of the drop containing oocytes just prior to immersion into liquid nitrogen (LN) is also very important. As previously reported, the concept of minimal drop size (MDS) of < 0.1 μl is critical not only for increasing the cooling rate but mainly for reducing the probability of ice nucleation [3, 4].

Finally, successful oocyte survival after vitrification is not attained if the warming process is not carefully considered. This step is very important and, as we have shown recently, the distance between the LN and the warming solution as well as the volume and the composition of the warming solution affects the warming rate and negatively impact the resulting oocyte survival [5]. The cooling and warming rates are important not only because of the probability of vitrification but also because of the chilling injury to which human oocytes are much more susceptible than embryos.

As pointed out by Tannus et al. [1], an automatic procedure is necessary for reducing the human factor and lack of reproducibility during vitrification and warming. Given the increasing utilization of this procedure, we firmly believe in a simpler (whether semi-manual or automatic) and standardized methodology as a crucial step for successful oocytes cryopreservation [6].

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