In vitro Human Embryo Culture; When Questions Outweigh Answers

More than five million babies have been born by the application of assisted conception methods throughout the world, and about 1% to 2% of babies in developed countries are born by the help of assisted reproductive techniques (ART) in couples with infertility. In spite of huge developments in equipments, techniques, procedures, supplies and greater control of environmental factors which affect assisted conception processes, there is mounting concern regarding anomalies and birth defects from assisted conception in the community and, especially, among infertile couples. Although this concern is very low compared to the time of application of IVF technology three decades ago, but results of more comprehensive longitudinal studies have revealed increased risk of congenital anomalies, malformation and early pregnancy loss associated with ART(1).

What really increases the defects, or whether these defects are transferred through parental gametes to the offspring or whether suboptimal *in vitro* conditions cause permanent changes in gametes or embryos that will influence the future life of IVF babies are not clearly known yet. Comparison of babies conceived through ART with babies of infertile/subfertile couples conceived without these techniques and those of fertile couples emphasize the role of optimization of assisted conception processes on their success rate and on the present and future health of these babies.

Several important factors influencing the quality and health of gametes and embryos in ART are the candidates themselves and the conditions, suboptimal for instance, under which ART processes are run. The factors also include conditions influencing controlled ovarian stimulation, gametes retrieval and processing, fertilization techniques and *in vitro* embryo cultures. Duration and condition of *in vitro* embryo culture have critical roles in IVF success rate and susceptibility of babies to different diseases in future. A large number of variables can affect development and quality of embryos during *in vitro* culture, including culture media, quantity of embryos, volume of each droplet, temperature, gas phase composition and quality, IVF laboratory air quality, culture ware/contact supplies, overlay oil, number and capacity of incubators, equipment validation and many other factors that need precise control during *in vitro* embryonic development (2).

Regarding the large number of variables influencing *in vitro* culture of embryos, a question is raised whether which of the aforesaid factors could be of more importance and need greater attention and whether we really could control all these variables precisely similar to the controlling systems in the fallopian tubes and the uterus. As an example among hundreds of factors influencing ART outcomes, is the culture medium. For the time being, two commercial sets of media are available in the market, one set is sequential culture media with philosophy of "back to the nature" and another set is one-step culture media with philosophy of "let the embryo select". There are large numbers of documents and papers on the benefits of one set over the other in the development of embryos and many studies have not even confirmed the results of previous ones (3).

The urge to design and perform studies in reputable institutes and publish the findings in high impact peer-review journals and the advertising push by companies to present their products, make it difficult for IVF clinics to select the best culture medium for *in vitro* development of embryos. On the other side already, most embryo development assessments are based on morphological criteria. Most changes in embryo micro-environment are not reflected on its morphology; therefore, it is time to change the assessment procedures and open the door for sub-cellular level of data integration methods such as genome, proteome, transcriptome, glycome and metabolome. In fact, we should consider the compensatory mechanisms and whole embryonic functions at different levels of changes in each parameter (4). Hence, our knowledge on embryo culture against the unknown is too negligible.

Recently, several studies have focused on the effects of physicochemical changes on the epigenetic status of cultured embryos, but their findings are preliminary and in some instances contradictory to one another (5). Therefore, to protect embryos and prevent harmful errors to their future health, we need to focus on the details of changes made to embryonic cultures and the compensatory functions of embryos towards those changes. But until the time we obtain these vital pieces of information, we should provide micro-environmental conditions as closely as possible to the womb conditions during *in vitro* embryonic cultures and this would not be possible except by implementing strict quality control/quality assurance programs.

References

1. Pinborg A, Henningsen AK, Malchau SS, Loft A. Congenital anomalies after assisted reproductive technology. Fertil Steril. 2013;99(2):327-32.
2. Lane M, Gardner DK. Embryo culture medium: which is the best? Best Pract Res Clin Obstet Gynaecol. 2007;21 (1):83-100.
3. Gruber I, Klein M. Embryo culture media for human IVF: which possibilities exist? J Turkish-German Gynecol Assoc. 2011;12:110-7.
4. Machtinger R, Racowsky C. Morphological systems of human embryo assessment and clinical evidence. Reprod Biomed Online. 2013;26(3):210-21.
5. El Hajj N, Haaf T. Epigenetic disturbances in in vitro cultured gametes and embryos: implications for human assisted reproduction. Fertil Steril. 2013 Jan 25. [Epub ahead of print].

Mohammad Reza Sadeghi
Editor-in-chief