9th Yazd International Congress and Student Award on Reproductive Medicine with 4th Congress of Reproductive Genetics

Award Winners

A-4

Gangliogenesis with folliculogenesis of ovary: Three-dimensional and two-dimensional analyses of Golgi-Cox-staining in mouse ovary

Asadi Zarch ME[1], Afshar A[2], Rahmanifar F[3], Jafarzadeh Shirazi MR[1], Baghban M[4], Dadpasand M[1], Mohammad Rezazadeh F[1], Khoradmehr A[2], Baharvand H[5, 6], Tamadon A[2].

1. *Department of Animal Sciences, College of Agriculture, Shiraz University, Shiraz, Iran.*
2. *The Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran.*
3. *Department of Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.*
4. *Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*
5. *Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.*
6. *Department of Developmental Biology, University of Science and Culture, Tehran, Iran.*

Email: amintamaddon@yahoo.com

Background: The ovarian follicular development of rodents begins at neonatal period, the stage at which primordial follicles are formed. During estrous cycle, most of the follicles undergoes atresia and some of them continue their development process. The mammalian's ovary is regulated by some factors including hormonal factors and direct neuron effects. Previous studies have shown that the fate of follicles in this cycle are affected by hormones such as follicle-stimulating hormone and luteinizing hormone. In addition, there are two different populations of neurons in ovary, the internal and external neurons. External nervous system of mouse ovary has many roles. Several studies have shown its role in developmental process, cyclic stages, pregnancy, and aging process. These nerves and also ganglia are responsible for ovarian estradiol secretion. Some studies implied that the ganglia in ovary takes part in some functions such as hormone secretion but to best of our knowledge, their relationship with follicular and ovarian development have not fully understood.

Objective: The present study was set out to investigate two-dimensional (2D) and three-dimensional (3D) evaluations of ovarian nervous network development and the structural relationship between folliculogenesis and gangliogenesis in mouse ovary.

Materials and Methods: Adult mice ovarian tissue samples were collected from diestrus and estrus stages. In details, firstly, the cardiac perfusion was performed. The collected ovarian samples were stained by a Golgi-Cox protocol. Following staining, tissues were serially sectioned with thickness of 30 μm for each section for imaging and further analysis. Ovarian tissue serial images were evaluated with Image J software for 2D analysis and with Imaris software for 3D analysis. The images of estrus and diestrus ovaries were separately compared. In addition, the 2D and 3D data of estrus ovary were comparably analyzed. IBM SPSS Statistics 26 software was used for statistical analysis. The mean differences between follicular groups were analyzed by one-way ANOVA and post hoc Tukey test.

Results: Neural filaments and ganglia were detected in the ovaries by Golgi-Cox staining. In both 2D and 3D studies, an increase in the number and area of ganglia was seen during the follicular growth ($p < 0.05$). The same pattern was also seen in corpora lutea development. However, in some cases such as ratio of ganglia number to follicle area, the ratio of ganglia area to follicular area, 2D findings were different compared with the 3D results. 3D analysis of ovarian gangliogenesis showed the possible direct effect of them on folliculogenesis. Golgi-Cox staining was used in this study for 3D evaluation in non-brain tissue. The results of 3D analysis of the present study showed that, in some cases, the information provided by 2D analysis does not match the reality of ovarian neuronal function. This confirmed the importance of 3D analysis for evaluation of ovarian function.

Conclusion: It was demonstrated that there was positive relationship between gangliogenesis and folliculogenesis in mouse ovary. Ovarian ganglia, as

an independent part of ovarian nervous system, is likely to have an important role in folliculogenesis and luteogenesis. Additionally, Golgi-Cox staining and 3D tissue imaging, instead of 2D imaging, are promising protocols for study of ganglia in ovarian tissue.

Key words: *3D Imaging, Ganglia, Ovarian follicle, Golgi-Cox staining, Mice.*

The original full text of this abstract has been published in scientific reports 2021; 11, 5547 (2021). https://doi.org/10.1038/s41598-021-84835-0.

How to cite to this article: *Asadi Zarch M.E., Afshar A., Rahmanifar F. et al. Three-dimensional and two-dimensional relationships of gangliogenesis with folliculogenesis in mature mouse ovary: a Golgi–Cox staining approach. Scientific Reports 2021; 11: 5547.*