Comment on "Structural changes in the rat placenta during the last third of gestation discovered by stereology"

We read with great interest the article by Šerman LJ et al. [1] about evaluation of the structural changes in the rat placenta during the last third of gestation, assessed by stereology. The authors concluded that the absolute volume of the whole placenta as well as the labyrinth had increased from day 16 to day 19 of gestation. In contrast, the volume density of glycogenic cells and trophoblast giant cells was higher on gestation day 16 than on day 19, probably due to the intensive trophoblast invasion during that time. Since stereological analysis assigns numerical values to analyzed structures, such outcomes ensure valid and exact comparison of healthy and pathologically altered tissue. In addition, comparison can be done between the tissues in different developing phases of an organ, as it was represented in this article.

However, we would like to highlight certain issues considering the methodology applied in this research. The authors conducted the research on a sample of six randomly selected placentas: three placentas collected at the day 16 and three placentas collected at the day 19 of gestation. From the total number (35) of collected rat placentas, it is not distinguishable how many inhered to each group and it is difficult to understand how they managed the random sampling. Although random sampling may be achieved in different ways (and each time it is possible to make an unbiased sampling and research), the results may vary in efficiency depending on the heterogeneity of the specimen itself [2]. The combination of optimally fixed tissue and the use of unbiased stereological techniques enable precise estimation of structural parameters to be obtained, allowing thus for highly statistically significant differences to be detected in relatively small sample size [3]. Most sampling procedures in stereological studies do not have fixed sample size. However, in order to prevent inconsistent results, each sampling level should be constant and the same average sample size should be used in calculation [4]. Systematic random sampling is declared more efficient than simple random sampling [5], and even more stereological estimates are based on grids of points and lines, which are essentially systematic sampling estimates. In our practice, following the basic

instructions for stereological analysis [3] of the human placenta, we implemented systematic random sampling at each stage of the research: in sampling of the selected placentas, blocks of placental tissue or tissue slides. However, respecting the fact that systematic random sampling leads to more precise results, we agree that this method is acceptable for a substantial amount of stereological research. Despite its limitations, this study represents a significant step towards gaining more clarity in this matter.

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