How organoids from endometrium and endometriosis could help to understand the pathogenesis of endometriosis

The article on methylation of *HOX* genes (1) in endometrium and endometriosis organoids is carefully performed and important research. Organoids, or the in vitro culture of cells as three-dimensional structures, are a rapidly developing new technology. The most important advantage of organoids is their phenotypic and genetic stability and long-lasting commitment to the tissue of origin during long-term culture (2). This characteristic was previously found only in specific immortalized monolayer cultured cell lines, which unfortunately differed from the original cells in many aspects.

Organoids thus begin to allow the study of in vitro of effects previously accessible only in vivo. Recently organoids were developed from isolated glandular-type fragments of endometrium, endometriosis, and endometrial cancers (3). These organoids likely develop from stem cells (Hugo Vankelecom, Leuven, personal communication). They form cell structures that mimic the endometrial glandular structure. These organoids could reproduce endocrine effects known during the menstrual cycle such as increasing mitoses induced by estrogens, the secretory changes induced by progesterone, and the effect of estrogen and progesterone withdrawal. The article by Esfandiari et al. (1) extends these observations by investigating *HOX* gene methylation and expression in epithelial organoids from endometrium and cystic ovarian endometriosis. In addition, organoids from endometriosis have a slightly different morphology.

The authors must be congratulated for their excellent work. However, the article also highlights the growing gap between basic research and the clinician. This gap needs an adapted writing style to bridge what is evident for the scientist and understandable for the clinician. As an example, the dispersion of endometrium into glands and stroma with the collection of glands through mesh filtration is a technique well known for more than 20 years. However, without prior knowledge it is difficult for the clinician to understand that the organoids described in this article consisted of epithelial cells only (1) without stromal cells. For the clinician, it is not clear to what extent the culture conditions might influence results.

Another difficulty is the growing gap between the many pathways described for the four *HOX* gene clusters and their many cofactors, their statistical cluster and pathway analysis, and the clinician's understanding of the relevant pathways. A comprehensive understanding is hampered by the complexity and redundancy of pathways. Also, the interpretation by the scientists in the discussion risks bias toward unsubstantiated clinical relevance (*hineininterpretierung* in German); although this may seem logical, it can also be viewed as close to speculation. The interpretation of *HOX* genes in organoids from glandular-type fragments to explain the pathophysiology of endometriosis is an example of this.

HOX genes are a group of genes that are evolutionary conserved and specify regions of the body plan of an embryo along the head–tail axis. They are a subset of homeobox genes that regulate transcription and control the cyclical endometrial development and receptivity. The article by Esfandiari et al. (1) confirms and extends the known differences in the expression of the four *HOX* clusters in the endometrium of women with endometriosis, possibly explaining the associated infertility. The article also broadens our understanding of these differences as epigenetic methylation changes of these genes and their regulator proteins or cofactors.

These investigators also further enhance our knowledge of the differences in *HOX* gene expression in ovarian endometriosis lesions, similar to the changes in the endometrium in 56 of 84 genes but contrariwise in the other 28. Although these differences may originate from the absence of stromal cells, as suggested by the authors, they may instead be due to additional epigenetic changes specific to initiate endometriosis that occur because of the hormone environment, the effect of oxidative stress, or another yet to be the determining cause.

The authors conclude that endometriosis organoids might be a "novel preclinical model to determine the epigenetic mechanisms that underlie endometriosis." Endometriosis organoids or endometrium organoids could theoretically allow the study of the induction of genetic changes and the induction and reversal of epigenetic changes in *HOX* gene methylation. However, this has yet to be demonstrated, and these studies need to consider the complexity and potential artifacts of the specific culture conditions to grow organoids and, in the actual organoids, the absence of interaction between endometrial epithelial and stromal cells, as pointed out by the authors in their discussion. In addition, these organoids lack blood vessels, innervation, and immune cells, which are needed for a complete model (2). It should additionally be emphasized that the organoids described were grown in the absence of sex steroid hormones and thus reflect *HOX* gene methylation of a not stimulated or growing endometrium or endometriosis.

These observed differences in methylation and *HOX* gene expression in endometriosis lesions and in the endometrium of women with endometriosis might be important in understanding the pathophysiology of endometriosis. It remains unclear whether and which changes in the endometrium of women with endometriosis are a consequence or a cause of endometriosis (4). It is tempting, as suggested by the authors, to use this organoid model to study methylation and *HOX* gene expression in the endometrium of adolescent daughters of women with severe endometriosis, who are known to have a much higher risk of developing (severe) endometriosis, but before these daughters develop endometriosis. According to the genetic-epigenetic theory (4), the hypothesis would be that some of these girls (i.e., those with a hereditary genetic or epigenetic predisposition) have changes in their endometrium similar to those found in women with endometriosis and that these changes will predict the probability of developing subsequent endometriosis. Because these organoids

can be cryopreserved, they will permit comparison of methylation and *HOX* gene expression of the endometrium before and after the development of endometriosis. An absence of differences would be an argument that the endometrial changes are preexisting to the development of endometriosis and that the associated infertility can be explained as a consequence of this genetic predisposition rather than of the endometriosis.

These organoids and the *HOX* gene methylation technology seem moreover suited to explore many other endometriosis and infertility problems that have remained intriguing for so long. A comparison of *HOX* gene methylation in organoids from endometrium before and after (severe) endometriosis surgery would permit the exploration of which endometrial changes are a reversible consequence of endometriosis, and eventually a consequence of the migration of endometriosis cells to the endometrium (5). It would be intriguing to evaluate how changes found in the endometrium compare between women with cystic ovaries and deep endometriosis (i.e., whether inherited endometrial changes have a predictive value for which type of endometriosis will develop). This technology could clarify in which women subtle endometriosis lesions are an intermittent physiological phenomenon instead of the early stages of severe pathology. Finally, if in these organoids methylation of *HOX* genes could be manipulated, we might have a model to study the pathophysiology of the initiation of endometriosis and subsequently of the growth of lesions (5).

Because organoids can develop from single cells, this model might help understand the relationship between genetic-epigenetic changes in a cell and their morphological appearance. For endometriosis, it might answer the question of whether the appearance of microscopical nests of endometriosis at a distance from or at the periphery of deep endometriosis nodule is pathology or whether the endometriosis nodule induces the morphology, and whether the underlying epigenetic changes are reversible and will return to normal when the stimulus is removed (5).

In conclusion, we congratulate the authors for excellent and stimulating research and *Fertility and Sterility* for bringing us such fundamental research in a clinical journal.

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https://doi.org/10.1016/j.fertnstert.2020.11.022

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