

**Dear Editor,**

The published article by Dr. Ai, et al. (1) on the secretion of vascular endothelial growth factor (VEGF) in a three-dimensional endometrial culture was of great surprise to me as this model had been earlier introduced by Fasciani, et al. (2) in 2003. This model was later developed by me (3, 4) and endometrial effects of different medications were evaluated in this model (5-7).

- Noting the endometrial tissue explants which are situated in a fibrin jell as extracellular matrix, gene expressions and growth factors secretions seem to mimic natural conditions, especially during the first few weeks which the primary endometrial tissue exists and has not been replaced by the new cell generations (2).

- VEGF secretion by human endometrium is not a new finding and it has been previously discussed (8, 9) which mars the justification for doing the study.

- The main problem of the undertaken study lies in the fact that it does not express the time of supernatant collection from the wells. Regarding the replacement of the culture media in three-day intervals in this model\_ nine times during the study \_ one cannot identify the time VEGF has been assayed (on the 3<sup>rd</sup>, 6<sup>th</sup>, ninth or other days?) Additionally, no measuring unit has been provided for the changes in the amount of secreted growth factor. Furthermore, endometrial stromal cells have been claimed to be the source of VEGF secretion but the authors should have considered endometrial epithelium (10) or even the neutrophils present in the endometrial stroma (11) as the probable sources.

- The authors have equaled their study with that of Fugii et al. (12) while the endometrium of patients with endometriosis differs from normal endometrium (13), a point which has been addressed in my previous articles (7).

- And needless to say that secretion of VEGF is regulated by sex hormones. Use of endometrial samples from patients with ovarian cysts seems to be another weakness of the study. Noticing the underlying hormonal disorders in these patients (14), the endometrial biopsies in Ai, et al study cannot be regarded as normal entities.

- There seems to be some incoherencies between the text and the references provided, e.g. for references 4, 5, 10 and 11 in the introduction and 23 in the discussion.

- The magnification of the figures does not seem

to be accurate and it seems a unique magnification has not been utilized.

- The definition provided for endometriosis does not seem to be precise as endometrial glands and stroma grow in the extrauterine space and not endometrial vasculature.

- Moreover, use of 0.15 M thrombin concentration needs to be justified by the corresponding author as earlier studies have utilized thrombin dissolved in 0.15 M NaCl for such a model (2-6).

- Finally, the first sentence of the conclusion which introduces the aforementioned study as a model for endometriosis cannot be accepted as such.

**References**

1. Ai J, Esfandiari N, Casper R. Secretion of vascular endothelial growth factor in a three-dimensional culture of human endometrium; an In-vitro model for endometriosis. *J Reprod Infertil.* 2009;10(2):95-100.
2. Fasciani A, Bocci G, Xu J, Bielecki R, Greenblatt E, Leyland N, et al. Three-dimensional in vitro culture of endometrial explants mimics the early stages of endometriosis. *Fertil Steril.* 2003;80(5):1137-43.
3. Khazaei M, Esfandiari N, Javed M, Gotlieb L, Casper RF. Successful three-dimensional culture of human secretory phase endometrium as an in vitro model for endometriosis. *Fertil Steril.* 2004;82 suppl 2:S164.
4. Khazaei M, Esfandiari N, Gotlieb L, Casper RF. Angiogenesis following three-dimensional culture of isolated human endometrial stromal cells. *Fertil Steril.* 2004;82 suppl 2:S61-2.
5. Montaseri A, Khazaei M, Ghorbani R, Rezaei M. Evaluating the effects of Atorvastatin on cultured human endometrium in a three-dimensional fibrin matrix. *J Reprod Infertil.* 2007;7(4):385-66.
6. Esfandiari N, Khazaei M, Ai J, Bielecki R, Gotlieb L, Ryan E, et al. Effect of a statin on an in vitro model of endometriosis. *Fertil Steril.* 2007;87(2):257-62.
7. Khazaei M, Montaseri A, Casper RF. Letrozole stimulates the growth of human endometrial explants cultured in three-dimensional fibrin matrix. *Fertil Steril.* 2009;91(5 Suppl):2172-6.
8. Torry DS, Holt VJ, Keenan JA, Harris G, Caudle MR, Torry RJ. Vascular endothelial growth factor expression in cycling human endometrium. *Fertil Steril.* 1996;66(1):72-80.
9. Moller B, Rasmussen C, Lindblom B, Olovsson M. Expression of the angiogenic growth factors VEGF,

- FGF-2, EGF and their receptors in normal human endometrium during the menstrual cycle. *Mol Hum Reprod.* 2001;7(1):65-72.
10. Niklaus AL, Aberdeen GW, Babischkin JS, Pepe GJ, Albrecht ED. Effect of estrogen on vascular endothelial growth/permeability factor expression by glandular epithelial and stromal cells in the baboon endometrium. *Biol Reprod.* 2003;68(6):1997-2004.
  11. Gargett CE, Rogers PA. Human endometrial angiogenesis. *Reproduction.* 2001;121(2):181-6. Review.
  12. Fujii EY, Nakayama M, Nakagawa A. Concentrations of receptor for advanced glycation end products, VEGF and CML in plasma, follicular fluid, and peritoneal fluid in women with and without endometriosis. *Reprod Sci.* 2008;15(10):1066-74.
  13. Ulukus M, Cakmak H, Arici A. The role of endometrium in endometriosis. *J Soc Gynecol Investig.* 2006;13(7):467-76. Review.
  14. Lee MT, Bruot BC, Adams WC. Hormonal changes during the early development of ovarian cysts in the rat. *Biol Reprod.* 1986;35(3):542-8.

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