

**Expression of Vascular Endothelial Growth Factor  
(VEGF) Receptors: Comparative immunohistochemical  
study of prepubertal, reproductive and postmenopausal  
endometria of female albino rats**

Thesis

Submitted for Fulfillment of the  
M.Sc Degree in Histology

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2010

# Summary

Angiogenesis is an important process for development and differentiation of mammalian endometrium. These changes are necessary for implantation as well as for maintenance of pregnancy. Vascular Endothelial Growth Factor (VEGF) is a protein that has the potential to play a dynamic role in regulation of angiogenesis via interaction with its transmembrane tyrosine kinase receptors; Flt-1 and KDR. Sex hormones have a major role in controlling the expression of these receptors but there are a lot of conflicting reports about this role.

This work was carried out to study the immunoexpression of VEGF receptors in the endometrium (surface epithelium, glands, stromal cells and endothelium) at different ages and reproductive phases and to correlate them with the histological pictures in these phases.

Seventy female albino rats were included in this study. They were divided into three groups. The first group included ten rats in the prepubertal period (aged 4-6 weeks). The second group consisted of 50 rats in the reproductive period (pubertal age) whose ages ranged from 6-10 months. This group was further subdivided into five subgroups representing the four phases of estrous cycle and an early pregnancy period. Each subgroup included ten rats. The third group consisted of 10 rats in the postmenopausal period aged 15-18 months.

Vaginal smears were first done to group II (pubertal age group) to verify the phases of estrous cycle. All animals were sacrificed and the uteri were removed. Paraffin sections were subjected to Hx & E stain to examine the histological picture of the endometrium in the different age groups and different phases of the estrous cycle.

Sections were subjected to immunohistochemical stains, namely with monoclonal anti Flt-1 antibody and monoclonal anti KDR antibody. Reaction was visualized using a peroxidase-anti peroxidase detection kit, with DAB as a chromogen.

Morphometric measurements, using the Image Analyzer Computer System, were done to measure the mean uterine wall thickness and the mean optical density for VEGF receptors in the immunostained sections. The data obtained were statistically analyzed using ANOVA and t-tests.

Hx & E-stained prepubertal uterine sections revealed a thin uterine wall. In the pubertal age groups and throughout all phases of the estrous cycle, there was an increase in the thickness of the uterine wall. This was verified by histomorphometric measurements which revealed a statistically significant increase in uterine wall thickness in the pubertal age groups when compared to the prepubertal age (P value < 0.05). The greatest uterine thickness was detected in the diestrus phase.

The simple low columnar epithelial lining for the endometrial surface and glands in the prepubertal age changed into taller columnar partially ciliated in proestrus proceeding to pseudostratified columnar ciliated epithelium in the estrus metestrus, diestrus phases as well as during the early pregnancy period. Finally, the epithelium exhibited a cuboidal to a low columnar pattern in the postmenopausal age. Some of the endometrial epithelial and stromal cells appeared vacuolated with pyknotic nuclei suggesting apoptosis, during estrus, metestrus and diestrus phases.

Mitotic figures were detected in the epithelial lining during proestrus and metestrus. Stromal cells appeared compact with mitotic figures during proestrus. Leucocytic infiltration was reported during estrus and metestrus. During pregnancy,

a well-developed sub-epithelial capillary plexus was an important finding. Stromal cells appeared widely separated with vesicular nuclei suggesting a decidual reaction.

Immunostained prepubertal sections showed weak cytoplasmic immunoreactivity for Flt-1 and KDR in the surface epithelium and glands and a negative reaction in stromal and endothelial cells.

Strong immunoreaction for both receptors was reported during proestrus and estrus phases. During metestrus, moderate reaction for Flt-1 & KDR was observed in the surface epithelium and glands, moderate reaction in the stromal cells for Flt-1 and weak for KDR, while strong reaction in the endothelial cells for both VEGF receptors were found.

During diestrus, moderate immunoreactivity for Flt-1 was detected in the surface and glands, whereas a weak reaction was observed in the stromal and endothelial cells. On the other hand, weak immunoreactivity for KDR in the diestrus phase was observed at all examined sites.

In early pregnancy, the endometrium revealed moderate immunostaining for both receptors in surface epithelial, glandular, stromal and endothelial cells. Strong immunoreactivity for Flt-1 was detected in the embryonic cells of the blastocyst while the reaction appeared moderate for KDR.

The postmenopausal phase revealed a negative immunoreactivity for both Flt-1 and KDR in all examined cells of the endometrium.

## **Conclusion**

The current study revealed that the expression of VEGF receptors is highest in the pubertal age group with marked expression of these receptors in the proestrus phase followed by estrus phase and this supports the role of sex hormones especially estrogen hormone in regulating VEGF receptors expression. This study revealed also that Flt-1 receptor has the predominance in the expression in endometrial epithelial, stromal and blastocyst cells while KDR receptor has the superiority in the endometrial endothelial cells. In conclusion the up-regulation of Flt-1 and KDR could be involved in regulating the endometrial endothelial cell proliferation and increasing the endometrial vascular permeability especially at the implantation sites. This could lead to the prolongation of the lifespan of the endometrial cells aiming at maintaining a successful pregnancy especially in females suffering from habitual early abortions or from repeated failure of implantation in “in-vitro fertilization programs”