Immunohistochemical expression of vascular endothelial growth factor receptors Flt1 and KDR in the endometrium during the estrus cycle in albino rats

Amal M. Abbas[a], Mohamed S. Elgendy[b], Noha M. Afifi[a] and Asmaa M. Elsayed[b]

[a]Histology Department, Faculty of Medicine, Cairo University, Cairo and [b]Histology Department, Faculty of Medicine, Fayoum University, Fayoum, Egypt

Correspondence to Mohamed S. Elgendy, Histology Department, Faculty of Medicine, Fayoum University, 63514 Fayoum, Egypt
Tel: +20 100 539 6843; fax: +20 846 302 350; e-mail: drmohgendy@hotmail.com

Received 4 April 2013
Accepted 9 October 2013

The Egyptian Journal of Histology
2013, 36:823-836
77 (1416-2013)

Background
Angiogenesis is an important process in endometrial development and embryonic implantation and is regulated through vascular endothelial growth factor (VEGF); its receptors Flt1 and KDR.

Aim
This work aimed to study the immunoexpression of VEGF receptors (VEGF-Rs) in the endometrium at different ages and reproductive phases and correlate them with the histological profiles in these phases.

Materials and methods
Seventy female albino rats were included in this study. They were divided into seven groups of 10 rats each: one group consisted of rats in the prepubertal period at age 4–6 weeks; five groups consisted of rats in the reproductive period at age 6–10 months, which were divided according to estrus cycle phases into proestrus, estrus, metestrus, diestrus, and pregnant groups; and the sixth group consisted of rats in the postmenopausal period at age 15–18 months. The uteri of all rats were removed and processed for staining with H&E and were subjected to immunohistochemical staining for Flt1 and KDR. For morphometric measurements, uterine wall thickness and Flt1 and KDR optical density in the endometrial surface epithelium, glandular epithelium, stromal cells, and endometrial endothelial cells were measured using image analysis. Results were statistically compared.

Results
The expression of VEGF-Rs was highest in the pubertal age group with marked expression of these receptors in the proestrus phase followed by the estrus phase. This supports the role of sex hormones, especially the estrogen hormone, in regulating VEGF-R expression. The Flt1 receptor was predominantly expressed in endometrial and stromal cells as well as in blastocysts, whereas the KDR receptor was predominantly expressed in endometrial endothelial cells. Comparison among all groups and then between each two groups revealed statistically significant differences in the measured morphometric parameters.

Conclusion
The upregulation of Flt1 and KDR could be involved in the regulation of endometrial endothelial cell proliferation and in increase in endometrial vascular permeability, especially at implantation sites.

Keywords:
endometrium, estrus cycle, Flt1, KDR, vascular endothelial growth factor-receptor

Egypt J Histol 36:823-836

1110-0559

Introduction

Vascular endothelial growth factor (VEGF) is a proangiogenic growth factor that acts as a potent mitogen for vascular endothelial cells [1] and has been shown to act as a cytoprotective agent, protecting these cells from apoptosis [2]. VEGF exerts its cellular effects through interaction with its transmembrane tyrosine kinase receptors Flt1 (VEGF-R1) and KDR (VEGF-R2) [3]. KDR is the principal mediator of the angiogenic effect of VEGF and its importance is highlighted by failure of KDR-null mice to develop organized blood vessels, resulting in lethality between embryonic days 8 and 10 [4]. The role of Flt1 in angiogenesis is less apparent and is a subject of debate. Some studies postulated that, during early embryogenesis, Flt1 is important in vascular modeling, and Flt1-null mutants die at mid-gestation with vascular overgrowth and disorganization [5]. Some reports have indicated that Flt1 has limited signaling activity and may act as a decoy receptor [6], whereas other studies have implicated Flt1 in the

mediation of endothelial proliferation [7], chemotaxis [8], and cell survival [9]. Reports were conflicting with regard to the role of female sex hormones estrogen and progesterone in controlling angiogenesis. Angiogenesis is also tremendously important in early pregnancy for confirming successful implantation of mammalian embryos at the blastocyst stages into the uterine endometrium [10].

Aim

The present study was planned to investigate the functional relationship between VEGF-Rs Flt1 and KDR expression during the different phases of the estrus cycle in albino rats for proper understanding of the vascular remodeling and angiogenesis of the endometrium, monitored by histological and immunohistochemical studies.

Materials and methods

The original research was approved by the ethics committee at the Histology Department, Faculty of Medicine, Cairo University (Cairo, Egypt) and followed international ethics and regulations for animal research in laboratory applications [11].

Animals

Seventy female albino rats locally bred at the animal house of the Faculty of Medicine, Cairo University, were used in this study. The animals received a standard diet for rats and were allowed free access to water.

They were divided into seven groups, each consisting of 10 rats: one group comprised rats in the prepubertal period at age 4–6 weeks; five groups comprised rats in the pubertal reproductive period at age 6–10 months – the proestrus, estrus, metestrus, diestrus, and pregnant groups – and the seventh group comprised in the postmenopausal period at age 15–18 months. The rats were sacrificed and their uteri were removed, processed for paraffin sections, stained with H&E, and subjected to immunohistochemical staining for VEGF-Rs Flt1 and KDR.

Methods

Rats from all groups were anesthetized with thiopental sodium at a dose of 50 mg/kg injected subcutaneously before being sacrificed. A midline incision was made in each animal. The uterine horns were dissected and specimens were processed for paraffin sections. Serial sections of 7-μm thickness were cut and subjected to the following stains:

(1) H&E to examine the histological profile of the endometrium in the different age groups and in different phases of the estrous cycle [12].
(2) Immunohistochemical staining of uterine sections for VEGF-R antirat monoclonal antibodies VEGF-R1 and VEGF-R2, known as Flt1 (kit RB-9049-R7) and KDR (kit RB-9239-R7), respectively, obtained from NeoMarkers, Lab Vision Corporation (Westinghouse, California, USA).

Immunohistochemical study

These antirat monoclonal antibodies are suitable for immunohistochemical staining of formalin-fixed paraffin-embedded sections using the avidin–biotin–peroxidase complex technique.

Sections were dewaxed in xylene, rehydrated, and pretreated with 3% hydrogen peroxide for blocking endogenous peroxidase activity. Microwave-assisted antigen retrieval was then performed for 20 min. Sections were incubated overnight at 40°C with the corresponding antibody. After washing with PBS, sections were incubated with biotinylated IgG and then with streptavidin–peroxidase conjugate. Sections were then washed with PBS and incubated with diaminobenzidine for 5 min and counterstained with Mayer's hematoxylin. Cells positive for Flt1 and KDR showed cytoplasmic brown deposits. Positive tissue control experiments were performed by immunostaining a section of rat lung for Flt-1-positive and KDR-positive cells. Negative control sections were obtained by omission of incubation with the primary antibody [13].

Quantitative morphometric analysis

The following parameters were examined using Leica Qwin 500 (Leica, Germany) image analysis:

(1) Uterine wall thickness measured using an objective lens of magnification ×4 – that is, a total magnification of ×40.
(2) Mean optical density for Flt1 and KDR in:
    (a) the endometrial surface epithelium;
    (b) endometrial glands;
    (c) endometrial stromal cells; and
    (d) endometrial endothelial cells.

Measurements were taken in five randomly selected nonoverlapping fields at a magnification of ×400 per slide from five slides of each animal.

Statistical analysis

Statistical analysis was performed on Excel software. Data are presented as mean ± SD. Differences among the study groups were detected using one-way analysis of variance as the global test to determine any differences in data before comparing pairs of groups and then using the *t*-test to compare between two groups. *P* values less than 0.05 were considered statistically significant [14].

Results

Histological results

H&E staining

Uterine sections of the prepubertal group showed a thin uterine wall with a narrow lumen and few short endometrial glands. The endometrial surface and endometrial glands were lined by simple low columnar epithelium. The underlying stromal cells were compact (Fig. 1).

Uterine sections of the proestrus group showed increased uterine wall thickness compared with the prepubertal group, with a narrow lumen lined by simple columnar

epithelium. There were few short endometrial glands lined with simple low columnar epithelial cells with vesicular nuclei. The underlying stromal cells were compact (Fig. 2).

The uterine wall of the estrus group was thick. The endometrial lining was pseudostratified columnar ciliated. There were tall endometrial glands that increased in size and became tortuous and lined with simple columnar epithelial cells. Some of the stromal cells showed vacuolations with pyknotic nuclei (Fig. 3).

In the metestrus group, uterine sections revealed a thick uterine wall. The endometrial lining was pseudostratified columnar ciliated. There were many tall and wide endometrial glands lined with pseudostratified columnar epithelial cells with vesicular nuclei. Some of the endometrial surface epithelial and glandular cells appeared vacuolated with fragmentation of chromatin material. Mitotic figures were seen in the underlying stromal cells (Fig. 4).

In the diestrus group the uterus showed a markedly thick wall. The endometrial lining was pseudostratified columnar ciliated. There were numerous tall endometrial glands. The endometrial glands were lined with simple or pseudostratified columnar cells with vesicular nuclei. Some cells with pyknotic nuclei were detected in surface epithelial and endometrial stromal cells (Fig. 5).

In the early pregnancy group, the uterine lumen was partially obliterated by the blastocyst. Patent parts of the uterine lumen appeared in between the blastocysts. The endometrium was lined by pseudostratified columnar ciliated epithelium. Well-developed subepithelial capillary plexus was present. Endometrial glands were lined with simple columnar cells. Stromal cells appeared rounded and widely separated (Fig. 6).

In the postmenopausal group, sections in the uterus showed marked reduction in the thickness of the uterine wall. The endometrium was lined by simple cuboidal or low columnar cells. Endometrial glands were scarce, extremely short, and lined with simple cuboidal or flattened cells (Fig. 7).

Immunohistochemical results

In the prepubertal group, endometrial surface epithelial cells and the glandular epithelium showed weak cytoplasmic Flt1 and KDR immunoreactivity. Endometrial stromal cells and endothelial cells showed negative reaction (Figs 8 and 9, respectively).

In the proestrus group, strong positive cytoplasmic Flt1 and KDR immunoreactivity was detected in endometrial surface epithelial cells, in the glandular epithelium, endometrial stromal cells, and endothelial cells (Figs 10 and 11, respectively).

In the estrus group, endometrial surface epithelial cells, glandular cells, endometrial stromal cells, and endothelial cells showed strong cytoplasmic Flt1 and KDR immunoreactivity (Figs 12 and 13, respectively).

In the metestrus group, moderate cytoplasmic Flt1 and KDR immunoreactivity was detected in the endometrial surface, glandular epithelium, and stromal cells. Endothelial cells showed strong cytoplasmic reaction (Figs 14 and 15, respectively).

In the diestrus group, endometrial surface epithelial cells and the glandular epithelium showed moderate cytoplasmic Flt1 immunoreactivity. Endometrial stromal cells and endothelial cells exhibited weak cytoplasmic reaction (Fig. 16).

In the diestrus group, weak cytoplasmic KDR immunoreactivity was encountered in surface epithelial endometrial cells, glandular epithelial cells, and endometrial stromal and endothelial cells (Fig. 17).

In the early pregnancy group, moderate cytoplasmic Flt1 immunoreactivity was detected in endometrial surface epithelial cells, glandular epithelial cells, endothelial cells, and surrounding stromal cells, whereas strong positive immunoreactivity was encountered in the embryonic cells of the blastocyst (Fig. 18).

In the early pregnancy group, endometrial surface epithelial cells and glandular epithelial cells, endometrial stromal cells, and endothelial cells showed moderate cytoplasmic KDR immunoreactivity. Embryonic cells of the blastocysts also exhibited moderate immunoreactivity (Fig. 19).

In the postmenopausal group, negative cytoplasmic Flt1 and KDR immunoreactivity was encountered in the endometrial surface epithelial cells, glandular epithelium, and endometrial stromal cells and endothelial cells (Figs 20 and 21, respectively).

Morphometric results

Analyses of variance among groups as regards uterine wall thickness and Flt1 and KDR optical density in the endometrial surface epithelium, glandular epithelium, stromal cells, and endometrial endothelial cells showed that, in each parameter, there was statistically highly significant variance among the groups ($P<0.05$).

Hence, comparison between two groups at a time with respect to these parameters was performed using the Student *t*-test and the results are summarized in Tables 1–3 and Histograms 1–3.

Table 1 and Histogram 1 summarize the results of the mean uterine wall thickness in different age groups.

The greatest uterine wall thickness was detected in the diestrus phase, whereas the least thickness was detected in the prepubertal group. Statistical analysis using the *t*-test showed statistically significant differences when comparisons were made between each two groups, except between the estrus and metestrus phase, which revealed statistically insignificant results.

Table 2 and Histogram 2 summarize the results of the Flt1 immunoreaction optical density in different areas.

As regards reaction in the endometrial epithelial cells, the density of reaction was highest in the proestrus group followed by the estrus group. The prepubertal group showed the mildest expression, whereas the postmenopausal group showed negative results.

Statistical analysis using the *t*-test showed statistically significant differences when comparisons were made between two groups, except between the proestrus and estrus groups, which revealed no statistical significance.

As regards endometrial glandular cells, the mean optical density peaked in the proestrus phase followed by the estrus phase. The mildest expression was documented in the prepubertal age group. The postmenopausal age group revealed negative results.

The comparison between two groups revealed statistically significant differences, except between the estrus and proestrus phases.

As regards endometrial stromal cells, the density of reaction was highest in the proestrus phase followed by the estrus phase. The diestrus phase showed the mildest expression. The prepubertal and postmenopausal groups showed negative results.

Statistical analysis using the *t*-test showed statistically significant differences when comparisons were made between two groups, except between the following pairs, for which no statistical significance was observed:
(1) Proestrus and estrus phase.
(2) Metestrus and diestrus phase.

As regards endometrial endothelial cells the highest expression was detected in the proestrus phase followed by the estrus phase. The diestrus group revealed the mildest expression, whereas the prepubertal and postmenopausal group showed negative results. Statistical analysis using the *t*-test showed statistically significant differences between two groups, except between the estrus and metestrus phases, which revealed statistically insignificant results.

Table 3 and Histogram 3 reveal the results of KDR optical density in different areas of the studied groups.

As regards endometrial surface epithelial cells, the greatest expression was detected in the proestrus phase, followed by the estrus phase. The diestrus phase showed the mildest expression. The postmenopausal group showed negative results. Statistical analysis using the *t*-test showed statistically significant differences between two groups.

As regards endometrial glandular cells, the mean optical density peaked in the proestrus phase, followed by the estrus phase. The mildest expression was documented in the diestrus phase, whereas negative expression was noted in the postmenopausal age group.

Statistical analysis revealed statistically significant results on comparison between two groups.

As regards endometrial stromal cells, the highest expression was detected in the proestrus phase, followed by the estrus phase. The diestrus phase showed the mildest expression. Prepubertal and postmenopausal groups showed negative results. Statistical analysis using the *t*-test showed statistically significant results between two groups, except between the proestrus and estrus phases, which gave statistically insignificant results.

As regards endometrial endothelial cells, the greatest expression was detected in the proestrus and estrus phases. The diestrus phase showed the mildest expression. Prepubertal and postmenopausal groups showed negative results. Statistical analysis using the *t*-test showed statistically significant results between two groups, except between the proestrus and estrus phases, which showed statistically insignificant differences.

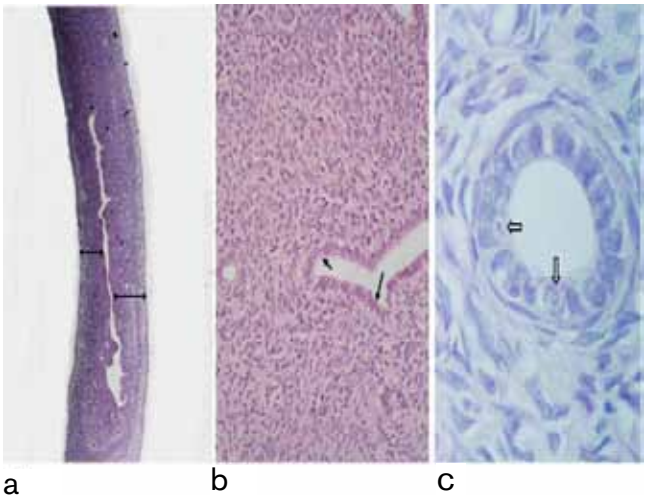


Figure 1. Photomicrograph of uterine sections from the prepubertal group showing a thin uterine wall (double headed arrows) with a narrow lumen and few short endometrial glands. The endometrial surface (thin arrows) and endometrial glands (thick arrows) are lined by simple low columnar epithelium. The underlying stromal cells are compact.
H&E, (a) × 40, (b) × 400, (c) × 1000.

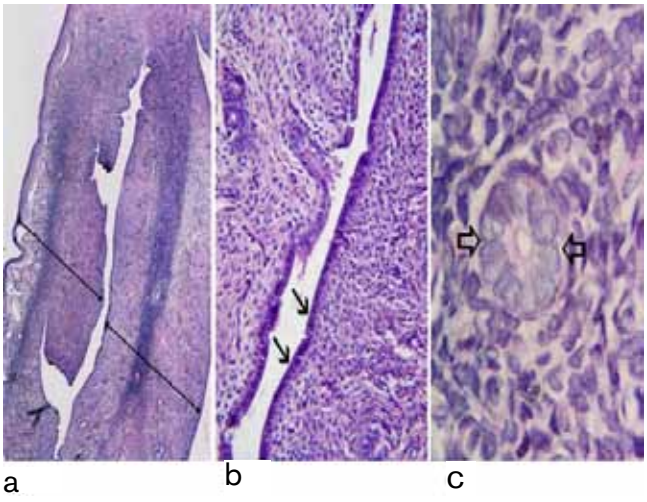


Figure 2. Photomicrograph of uterine sections from the proestrus group showing increased wall thickness (double headed arrows) with a narrow lumen lined by simple columnar epithelium (thin arrows). There are few short endometrial glands lined with simple low columnar epithelial cells with vesicular nuclei (thick arrows). The underlying stromal cells are compact.
H&E, (a) × 40, (b) × 400, (c) × 1000.

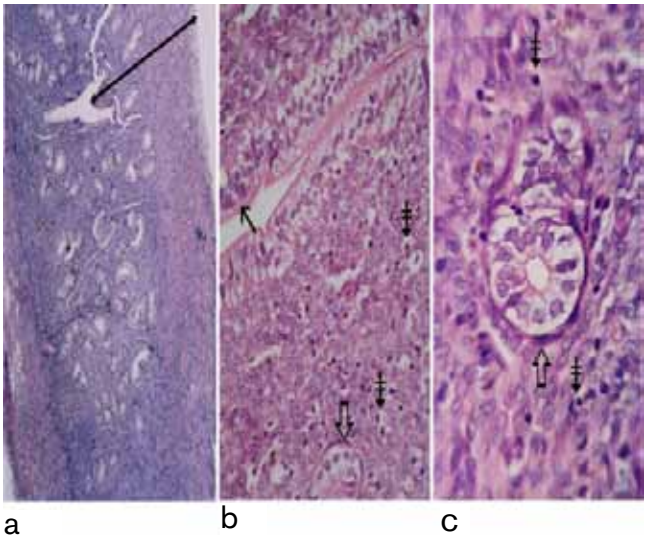


Figure 3. Photomicrograph of uterine sections from the estrus phase group showing a thick uterine wall (double headed arrow). The endometrial lining is pseudostratified columnar ciliated (thin arrow). The endometrial glands are lined with simple columnar epithelial cells (thick arrows) with pale nuclei and prominent nucleoli. Some of the stromal cells show vacuolations with pyknotic nuclei (crossed arrows), suggesting apoptosis.

H&E, (a) × 40, (b) × 400, (c) × 1000.

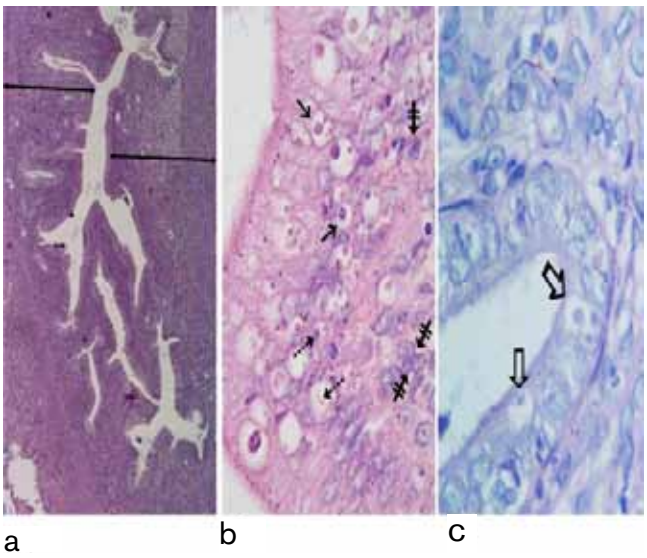


Figure 4. Photomicrograph of sections of the uterus from the metestrus phase group revealing a thick uterine wall (double headed arrows). The endometrial lining is pseudostratified columnar ciliated, containing many vacuolated cells with pyknotic nuclei (thin arrows). There are many endometrial glands with wide lumina lined with pseudostratified columnar epithelial cells with vesicular nuclei; however, some cells appear vacuolated (thick arrows) and some cells show fragmentation of chromatin material (dotted arrows). Mitotic figures are seen in the underlying stromal cells (crossed arrows).

H&E, (a) × 40, (b) × 1000, (c) × 1000.

Figure 5. Photomicrograph of sections of the uterus from the diestrus phase group showing a markedly thick wall (double headed arrow). The endometrial lining is pseudostratified columnar ciliated (thin arrows). There are numerous endometrial glands lined with pseudostratified columnar cells with vesicular nuclei (thick arrows). Some cells with pyknotic nuclei are detected in the surface epithelial and stromal cells (crossed arrows).

H&E, (a) × 40, (b) × 400, (c) × 1000.

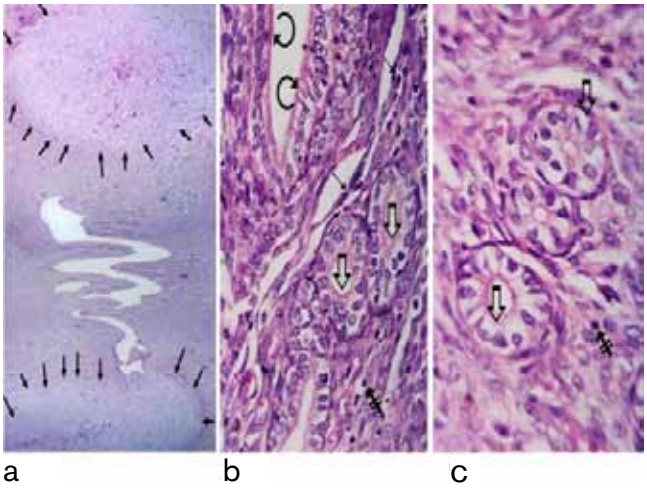


Figure 6. Photomicrograph of sections of the uterus from the early pregnancy group showing partially obliterated uterine lumen by blastocysts (thin arrows). Patent parts of the uterine lumen appear in between the blastocysts. The endometrium is lined by pseudostratified columnar ciliated epithelium (curved arrows). Well-developed subepithelial capillary plexus is present (dotted arrows). Endometrial glands are lined with simple columnar cells (thick arrows). Stromal cells appear rounded and widely separated (crossed arrows), suggesting decidual reaction.

H&E, (a) × 40, (b) × 400, (c) × 1000.

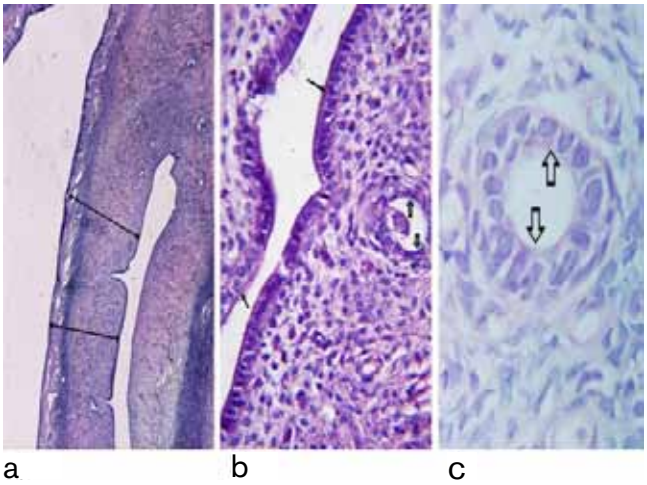
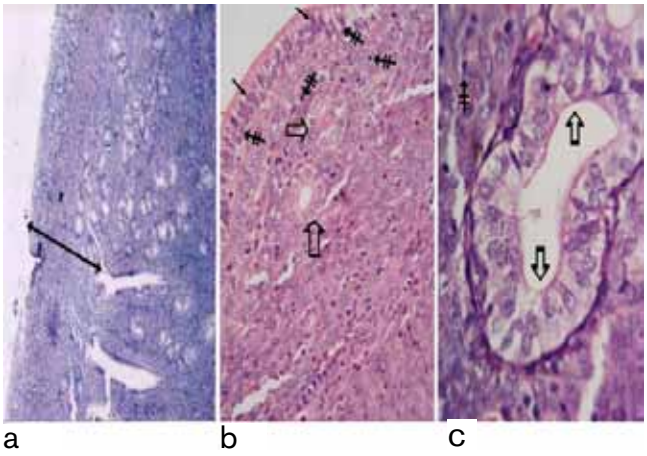


Figure 7. Photomicrograph of sections of the uterus from the postmenopausal group showing marked reduction in the thickness of the uterine wall (double headed arrows) compared with all pubertal phase groups. The endometrium is lined by simple cuboidal or low columnar cells (thin arrows). Endometrial glands are scarce, extremely short, and lined with simple cuboidal epithelium (thick arrows).

H&E, (a) × 40, (b) × 400, (c) × 1000.



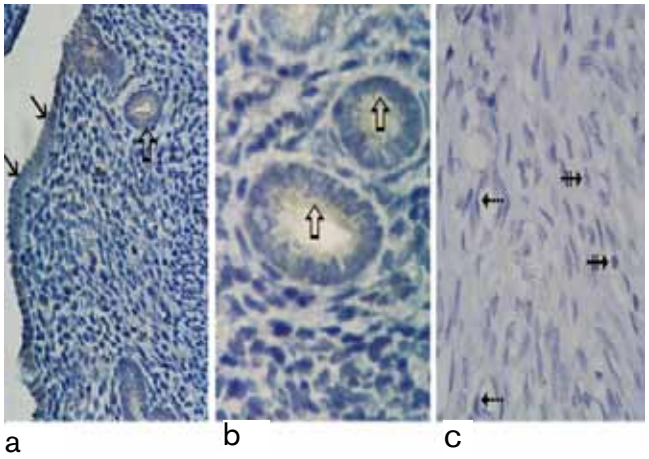


Figure 8. Photomicrograph of sections of the uterus from the prepubertal group showing endometrial surface epithelial cells (thin arrows) and glandular epithelium (thick arrows) with weak cytoplasmic Flt1 immunoreactivity. Endometrial stromal cells (crossed arrows) and endothelial cells (dotted arrows) show negative reaction.
Flt1 immunostaining, (a) × 400, (b) × 1000, (c) × 1000.

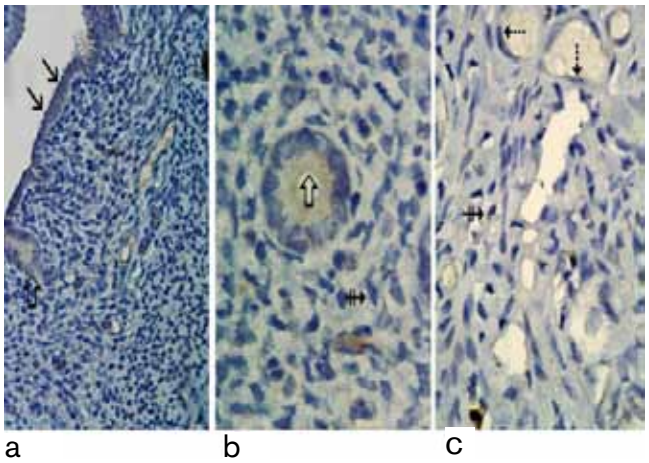


Figure 9. Photomicrograph of sections of the uterus from the prepubertal group showing endometrial surface epithelial cells (thin arrows) and glandular epithelium (thick arrows) with weak cytoplasmic KDR immunoreactivity. Endometrial stromal cells (crossed arrows) and endothelial cells (dotted arrows) show negative reaction.
KDR immunostaining, (a) × 400, (b) × 1000, (c) × 1000.

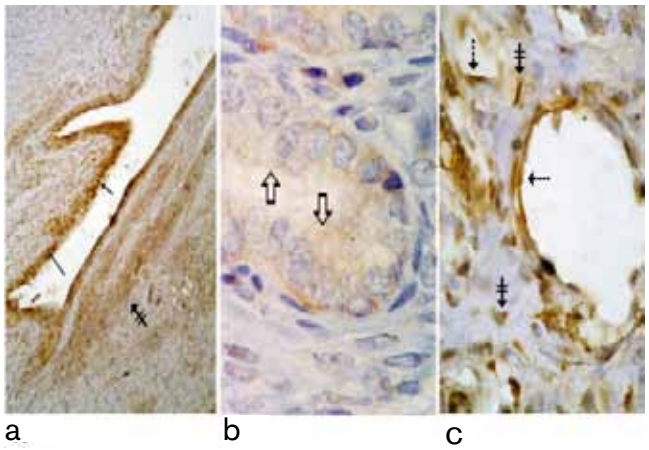


Figure 10. Photomicrograph of sections of the uterus from the proestrus group showing strong positive cytoplasmic Flt1 immunoreactivity in endometrial surface epithelial cells (thin arrows), glandular epithelium (thick arrows), endometrial stromal cells (crossed arrows), and endothelial cells (dotted arrows).
Flt1 immunostaining, (a) × 400, (b) × 1000, (c) × 1000.

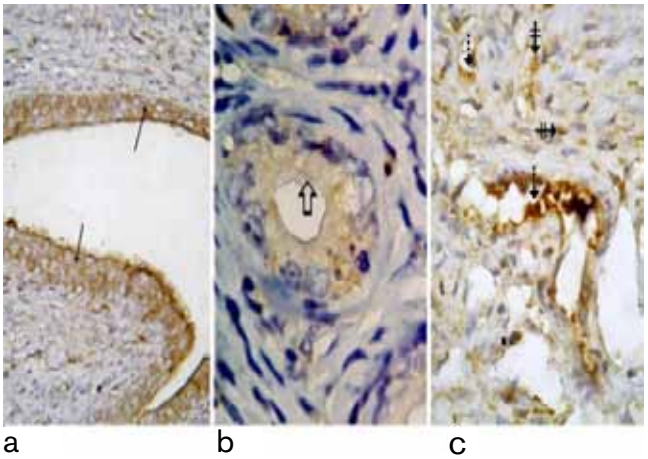


Figure 11. Photomicrograph of sections of the uterus from the proestrus group showing strong positive cytoplasmic KDR immunoreactivity in the endometrial surface epithelial cells (thin arrows), glandular epithelial cells (thick arrow), endometrial stromal cells (crossed arrows), and endothelial cells (dotted arrows).
KDR immunostaining, (a) × 400, (b) × 1000, (c) × 1000.

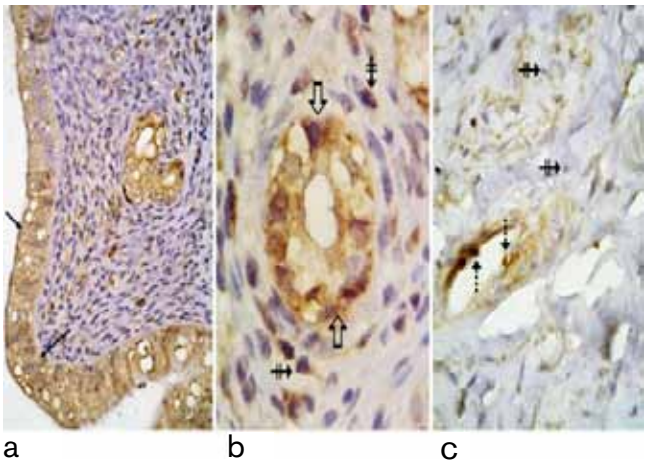


Figure 12. Photomicrograph of sections of the uterus from the estrus group showing endometrial surface epithelial cells (thin arrows), glandular cells (thick arrows), endometrial stromal cells (crossed arrows), and endothelial cells (dotted arrows) with strong cytoplasmic Flt1 immunoreactivity.
Flt1 immunostaining, (a) × 400, (b) × 1000, (c) × 1000.

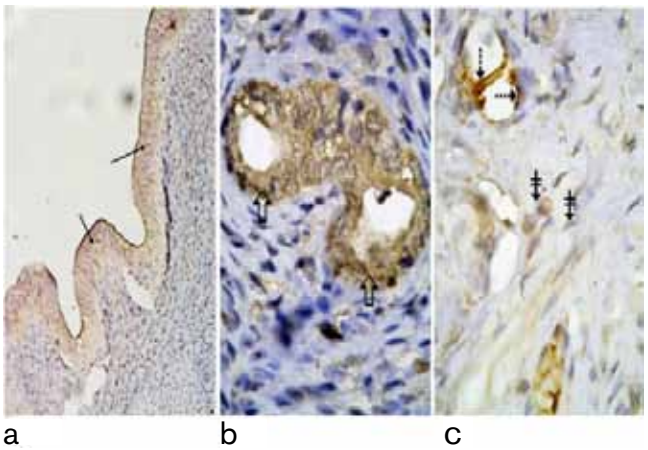


Figure 13. Photomicrograph of sections of the uterus from the estrus group showing strong cytoplasmic KDR immunoreactivity in endometrial surface epithelial cells (thin arrows), glandular cells (thick arrows), endometrial stromal cells (crossed arrows), and endothelial cells (dotted arrows).
KDR immunostaining, (a) × 400, (b) × 1000, (c) × 1000.

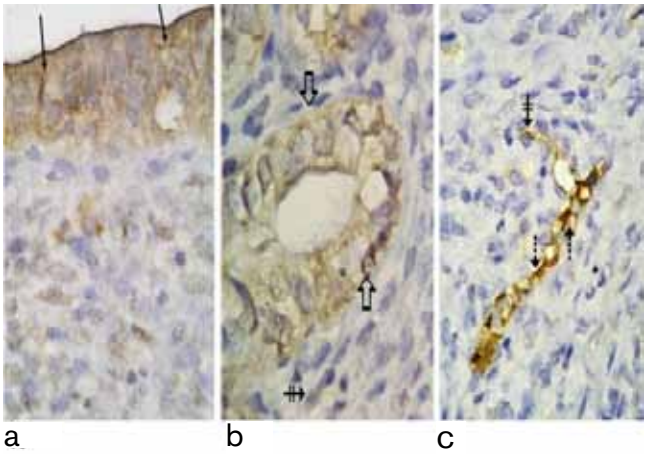


Figure 14. Photomicrograph of sections of the uterus from the metestrus group showing moderate cytoplasmic Flt1 immunoreactivity in the endometrial surface (thin arrows), glandular epithelium (thick arrows), and stromal cells (crossed arrows). Endothelial cells show strong cytoplasmic reaction (dotted arrows).
Flt1 immunostaining, (a) × 1000, (b) × 1000, (c) × 1000.

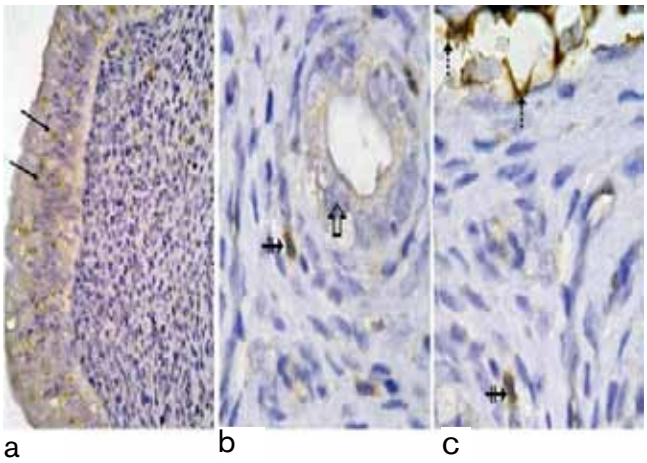


Figure 15. Photomicrograph of sections of the uterus from the metestrus group showing moderate cytoplasmic KDR immunoreactivity in the endometrial surface (thin arrows), glandular epithelium (thick arrow), and stromal cells (crossed arrows). Endothelial cells show strong cytoplasmic reaction (dotted arrows).
KDR immunostaining, (a) × 400, (b) × 1000, (c) × 1000.

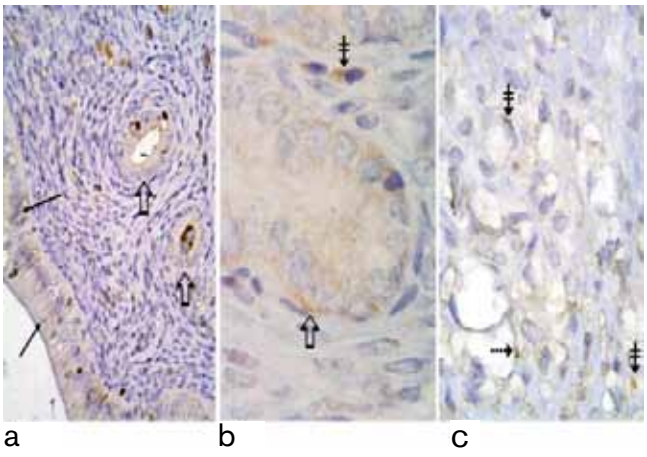


Figure 16. Photomicrograph of sections of the uterus from the diestrus group showing moderate cytoplasmic Flt1 immunoreactivity in endometrial surface epithelial cells (thin arrows) and glandular epithelium (thick arrows). Endometrial stromal cells (crossed arrows) and endothelial cells (dotted arrow) show weak cytoplasmic reaction.
Flt1 immunostaining, (a) × 400, (b) × 1000, (c) × 1000.

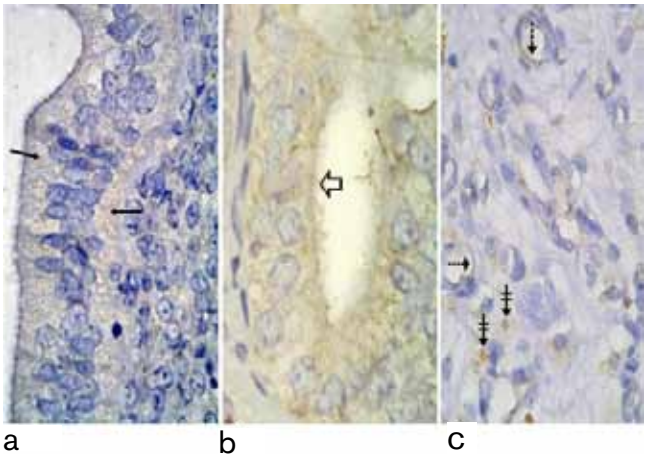


Figure 17. Photomicrograph of sections of the uterus from the diestrus group showing weak cytoplasmic KDR immunoreactivity in surface epithelial endometrial cells (thin arrows), glandular epithelial cells (thick arrow), endometrial stromal cells (crossed arrows), and endothelial cells (dotted arrows).
KDR immunostaining, (a) × 1000, (b) × 1000, (c) × 1000.

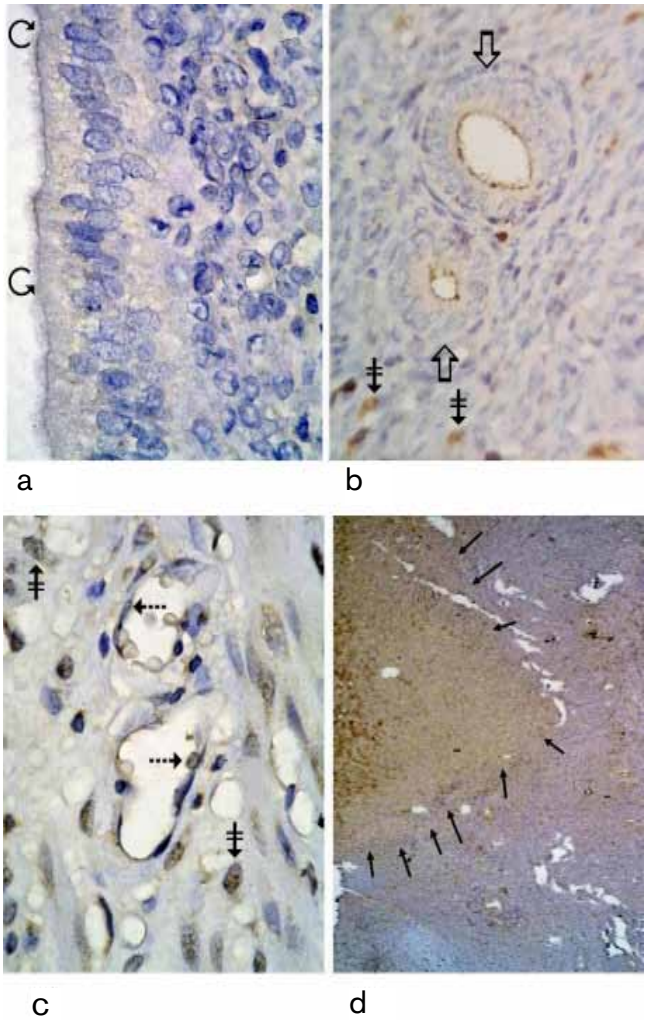


Figure 18. Photomicrograph of sections of the uterus from the early pregnancy group showing moderate cytoplasmic Flt1 immunoreactivity in the endometrial surface epithelial cells (curved arrows), glandular epithelial cells (thick arrows), endothelial cells (dotted arrows), and surrounding stromal cells (crossed arrows), and strong positive immunoreactivity in the embryonic cells of the blastocysts (thin arrows).
Flt1 immunostaining, (a) × 1000, (b) × 1000, (c) × 1000, (d) × 40.

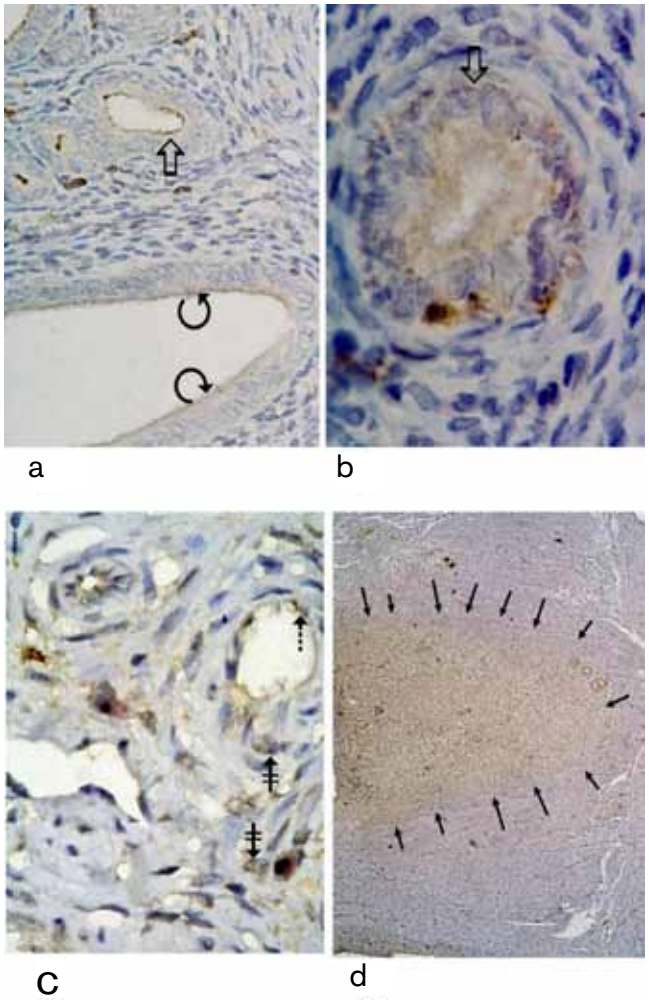


Figure 19. Photomicrograph of sections of the uterus from the early pregnancy group showing moderate cytoplasmic KDR immunoreactivity in the endometrial surface epithelial cells (curved arrows) and glandular epithelial cells (thick arrows), endometrial stromal cells (crossed arrows), and endothelial cells (dotted arrow). Embryonic cells of the blastocysts (thin arrows) also show moderate immunoreactivity.
KDR immunostaining, (a) × 400, (b) × 1000, (c) × 1000, (d) × 40.

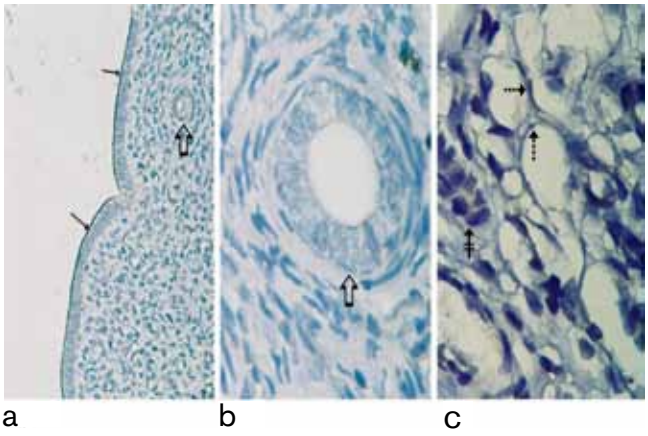


Figure 20. Photomicrograph of sections of the uterus from the postmenopausal group showing negative cytoplasmic Flt1 immunoreactivity in endometrial surface epithelial cells (thin arrows), glandular epithelium cells (thick arrows), and endometrial stromal cells (crossed arrow) and endothelial cells (dotted arrows).
Flt1 immunostaining, (a) × 400, (b) × 1000, (c) × 1000.

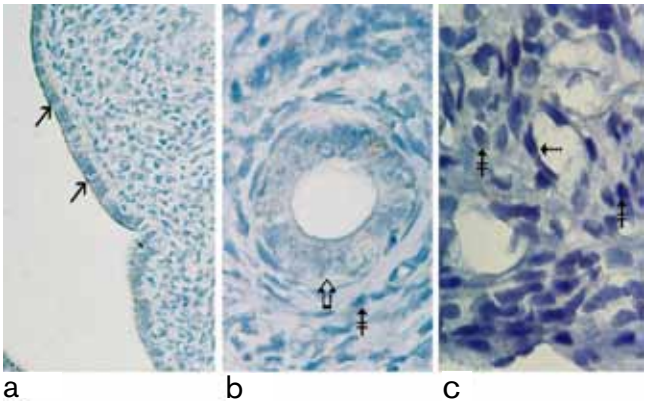
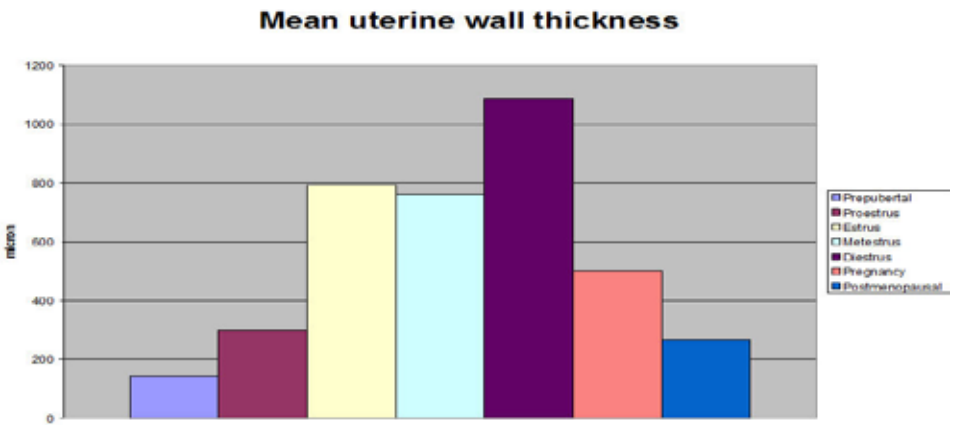


Figure 21. Photomicrograph of sections of the uterus from the postmenopausal group showing negative cytoplasmic KDR immunoreactivity in endometrial surface epithelial cells (thin arrows), glandular epithelium cells (thick arrow), and endometrial stromal cells (crossed arrows) and endothelial cells (dotted arrows).
KDR immunostaining, (a) × 400, (b) × 1000, (c) × 1000.

Table 1. Mean uterine wall thickness (µm)

Mean ± SD	Prepubertal	Proestrus	Estrus	Metestrus	Diestrus	Pregnancy	Postmenopausal
Uterine wall thickness	142.839 ± 3.653[a]	299.711 ± 7.077[b]	792.571 ± 58.339[c]	761.252 ± 21.926[d]	1087.031 ± 40.773[e]	501.156 ± 17.254[f]	266.708 ± 11.057[g]

Superscripts indicate statistically significant differences ($P<0.05$) compared with other groups.

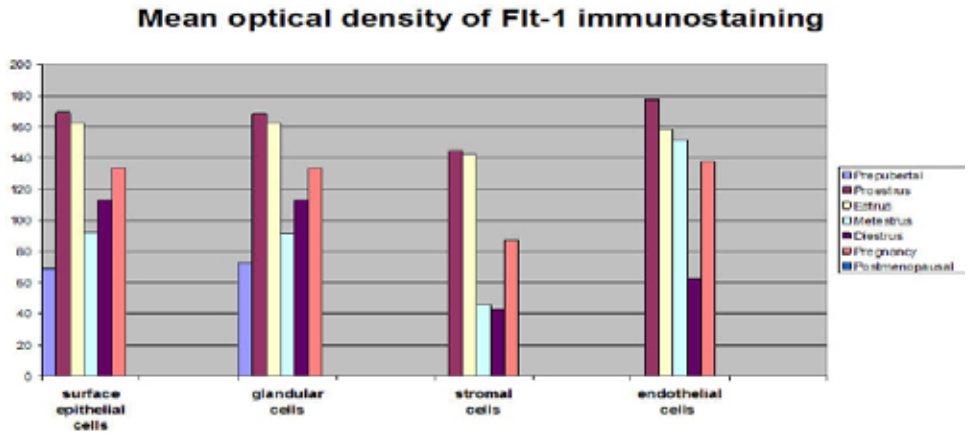


Histogram 1. Mean uterine wall thickness in the groups.

Table 2. Mean optical density of Flt1 immunostaining

Mean ± SD	Prepubertal	Proestrus	Estrus	Metestrus	Diestrus	Pregnancy	Postmenopausal
Endometrial epithelial cells	69.456 ± 5.595[a]	168.81 ± 6.299[b]	163.036 ± 5.981[b]	92.45 ± 3.762[c]	112.85 ± 1.3401[d]	133.35 ± 1.787[e]	0[f]
Endometrial glandular cells	72.426 ± 9.74[a]	168.274 ± 6.31[b]	163.0888 ± 4.91[b]	91.424 ± 2.87[c]	112.98 ± 1.26[d]	133.222 ± 1.77[e]	0[f]
Endometrial stromal cells	0[a]	144.21 ± 3.882[b]	142.37 ± 1.972[b]	45.91 ± 3.7834[c]	42.73 ± 3.762[c]	87.37 ± 2.215[d]	0[a]
Endometrial endothelial cells	0[a]	177.637 ± 2.811[b]	158.286 ± 6.257[c]	151.906 ± 1.586[c]	62.408 ± 4.944[d]	137.447 ± 3.601[e]	0[a]

Superscripts indicate statistically significant differences ($P<0.05$) compared with other groups.

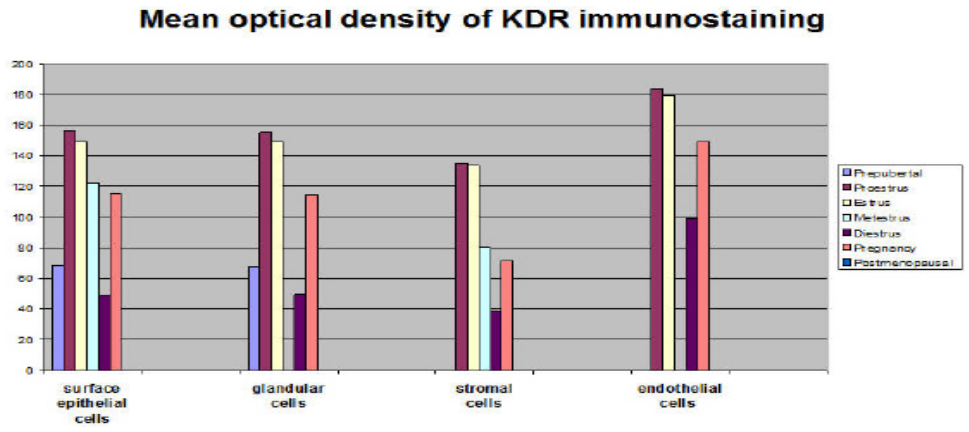


Histogram 2. Mean optical density of Flt1 immunostaining in the groups.

Table 3. Mean optical density of KDR immunostaining

Mean ± SD	Prepubertal	Proestrus	Estrus	Metestrus	Diestrus	Pregnancy	Postmenopausal
Endometrial epithelial cells	68.42 ± 8.411[a]	156.09 ± 2.468[b]	149.155 ± 2.5499[c]	122.14 ± 4.312[d]	49.42 ± 1.4718[e]	114.86 ± 2.761[f]	0[g]
Endometrial glandular cells	67.437 ± 7.63[a]	155.38 ± 2.29[b]	148.934 ± 2.58[c]	121.847 ± 4.14[d]	49.481 ± 0.78[e]	114.729 ± 2.16[f]	0[g]
Endometrial stromal cells	0[a]	134.932 ± 2.758[b]	133.486 ± 2.031[b]	80.31 ± 0.831[c]	39.18 ± 6.734[d]	71.65 ± 1.223[e]	0[a]
Endometrial endothelial cells	0[a]	183.808 ± 2.555[b]	179.412 ± 5.692[b]	159.147 ± 4.379[c]	99.277 ± 1.621[d]	149.34 ± 2.371[e]	0[a]

Superscripts indicate statistically significant differences ($P<0.05$) compared with other groups.



Histogram 3. Mean optical density of KDR immunostaining in the groups.

Discussion

Angiogenesis is the growth of new blood vessels from pre-existing vasculature. It plays a role in the monthly growth and regression of the human endometrium under the overall control of the ovarian steroids estrogen and progesterone [15].

VEGF is one of the major regulators of angiogenesis. It works by binding to high-affinity tyrosine kinase receptors. Two of the most important receptors are fms-like tyrosine kinase (Flt1, VEGF-R1) and the fetal liver kinase-1/kinase insert domain-containing region (Flk-1/KDR, VEGF-R2) [16–18].

In this study we examined the histological characteristics of the endometrium, and measured the intensities of immunostaining for VEGF-Rs Flt1 and KDR in different groups in the prepubertal, pubertal, and postmenopausal age groups and in the different phases of the estrous cycle in female albino rats to correlate histological criteria with the age and cycle phase in each group.

Significant increase in uterine wall thickness was observed in the pubertal age groups when compared with the prepubertal age group. During the estrus cycle there was variation in thickness according to the estrus phase. In the prepubertal group of the current study, the endometrial surface was lined with simple low columnar cells. Few short endometrial glands lined with cuboidal epithelium were seen, which could be explained by the low level of estradiol during this phase of development, as reported in other studies [19,20].

The proestrus phase revealed the least thickness during the phases of the estrous cycle. The endometrium was lined by simple columnar partially ciliated cells with underlying compact stroma. During the estrus stage, the uterine lumen was lined with pseudostratified columnar ciliated epithelium. Many of the surface and glandular epithelial cells exhibited vacuolar degeneration with pyknotic nuclei. This might be explained by apoptotic changes in the uterine components due to decrease in estradiol levels. These results are in accordance with other studies correlating low estrogen level during the estrus phase with the decrease in glandular and luminal epithelial proliferation and increased apoptosis in these cells [21].

Menstruation is absent in rodents; hence, apoptosis and regenerative factors aid in maintaining endometrial integrity. The highest apoptotic expression occurs during the estrus phase [22,23].

In the metestrus phase the endometrial surface and glandular cells were lined by pseudostratified columnar epithelium. Both the endometrial epithelial and stromal cells in this phase showed vacuolar degeneration. These findings are in agreement with other studies correlating these changes to both E2 and P4 levels, which are relatively low at this stage. Mitotic figures were also observed in the lining epithelium [24,25].

The uterine wall revealed the greatest thickness in the diestrus phase, followed by estrus and metestrus phases. This observation differed from other reports, in which the greatest uterine width was seen in the estrus phase, followed by the metestrus, diestrus, and proestrus phases. This difference might be related to the way in which the uterine wall thickness was measured: we measured it from the inner limit of the endometrium to the outer limit of the perimetrium on the same uterine wall, whereas others measured it from the perimetrium on one side to the perimetrium on the other side [19].

During the diestrus phase, endometrial lining epithelium was pseudostratified columnar. Some of the glands were lined with simple columnar cells and others with pseudostratified columnar epithelium. Some of the endometrial and stromal cells showed pyknotic nuclei, probably apoptotic cells. This finding might correlate with the low level of estrogen in this phase. During early pregnancy, the uterine lumen was partially obliterated by blastocysts and remained patent between the blastocysts. Endometrial stromal cells appeared widely separated and rounded, with vesicular nuclei and prominent nucleoli, suggesting a decidual reaction and odematous stroma. These histological findings are similar to those seen in the pregnant human uterus with the exception of different implantation sites and different time courses [26].

The presence of some apoptotic cells with pyknotic nuclei was reported during pregnancy in both glandular epithelial cells and stromal cells. This is in agreement with the reports of other investigators who observed apoptotic cell death in the rat endometrium during early pregnancy [27].

Indeed, apoptosis is presumably an important part of the process of uterine remodeling before implantation of blastocysts. It has been well recognized that the rat uterine epithelium cyclically proliferates and undergoes regression during the estrus cycle. Well-developed subepithelial capillary plexus was reported in the pregnant endometrial sections of the present study as in many other studies [28].

In the present study, the postmenopausal uterine group showed regression in the uterine wall thickness when compared with other groups. The endometrial epithelium was lined by cuboidal to low columnar epithelial cells with condensed nuclei. The few encountered endometrial glands were lined with flat or cuboidal cells. This was the basic picture of the postmenopausal endometrium [29].

Angiogenesis is essential for mammalian endometrial development, helping in implantation and maintenance of pregnancy. An important regulator of angiogenesis is known as VEGF. It acts by binding to its transmembrane tyrosine kinase receptors Flt1 and KDR [30,31].

In the present work, the intensity of immunostaining of VEGF-Rs was examined in surface endometrial epithelial cells, glandular cells, stromal cells, and endothelial cells of the blood vessels in different age groups and different phases of the estrous cycle.

In this study, morphometric measurements showed that the highest intensity of reaction in surface epithelial,

glandular, stromal, and endothelial cells of the endometrium was found in the proestrus phase of the cycle. No reaction was encountered for either receptor in any of the examined sites in the postmenopausal age group.

In the prepubertal group, weak reaction was seen for both Flt1 and KDR in the surface epithelial cells and glandular cells. Negative reaction was seen in stromal and endothelial cells of blood vessels. No previous studies examining VEGF-Rs in the prepubertal age group could be found to compare with the results in the present study. The negative reaction in endometrial stromal and endothelial cells for both Flt1 and KDR is probably due to the absence of sex steroid hormones, which regulate the expression of VEGF-Rs. The weak reaction in the endometrial epithelium for both Flt1 and KDR may be due to the requirements of the growth process that occurs in this age group [32].

On comparing the intensity of immunoreaction in the different phases of the estrous cycle in the pubertal age group it was found that the peak value for both receptors was always encountered in the proestrus phase, followed by the estrus phase, at all examined sites. The mildest intensity for Flt1 in surface epithelial and glandular cells was observed in the metestrus phase and for the stromal and endothelial cells in the diestrus phase. However, the mildest reaction for KDR at all examined sites was detected in the diestrus phase, whereas no remarkable reaction was seen in the postmenopausal group as endometrial proliferation was due to estrogen secretion [33].

It was also observed in the present work that KDR immunoreactivity in the endothelial cells was higher than that for Flt1. This might be explained by the fact that KDR is the main signaling receptor in the endothelium [34,35].

After implantation, KDR expression decreases to moderate values and is maintained by the effect of E2 and P4 secreted by the corpus luteum. This small decrease in vascularity may be essential to retain the implanted ovum in the endometrium without immunological rejection. If pregnancy does not occur, the diestrus phase follows with the presence of only P4, and this leads to decreased angiogenesis and the degenerative and quiescent state of the endometrium [36].

In all age groups examined in this study the immunoreactivity for VEGF-Rs was higher in the epithelium than in the stroma. This might be explained by the fact that the epithelium is devoid of any blood vessels, and epithelial cells are polarized and separated from the stroma by a basement membrane; hence, it seems reasonable to assume that the epithelium is more hypoxic than the stroma and hypoxia stimulates angiogenesis [32].

It was also observed in this study that in the epithelial and stromal cells the immunoreactivity for Flt1 was slightly higher than the immunoreactivity for KDR. The exact reason for this is not well understood, as the precise function of Flt1 is still under debate. Functions and signaling properties can differ depending on the developmental stage and cell type, as in endothelial and nonendothelial cells [37].

Results of this study support the role of sex hormones in regulation of the expression of VEGF-Rs, which is mainly stimulated by estrogen and to a lesser extent by progesterone. This is in agreement with other investigators who observed foci of VEGF immunostaining in the endometrial stromal compartment, particularly during the proliferative stage, and most of this VEGF was associated with blood vessels [38].

Estrogen exerts its action on estrogen-responsive elements, resulting in an increase in transcriptional activity. Hence, estrogen stimulates angiogenesis by acting both directly on endothelial cells and/or indirectly on other endometrial cell types through numerous potential promoters [39].

In the current work, during the early pregnancy period (2–6 days of pregnancy), moderate epithelial, endothelial, and stromal immunostaining for both Flt1 and KDR was observed. Embryonic cells showed higher density of immunostaining compared with the surrounding stromal cells. These findings are consistent with those of others who reported moderate to weak immunoreactivity to VEGF-Rs in the epithelial and stromal cells in pregnant rats [40,41].

However, there was strong VEGF expression in decidual cells and strong Flt1 and KDR expression in vascular endothelial cells, in addition to high proliferative activity including endothelial cell proliferation on day 5 of pregnancy in rats [42].

Previous studies have reported a significant increase in vascular permeability at the implantation sites relative to the myometrium and nonimplanted sites of the uteri. The blood volume increase was significant on day 7 in rats. Vascular development during embryo implantation is mainly governed by the VEGF [36].

VEGF exerts its vasodilatory effects through activation of endothelial nitric oxide synthase and through prostacyclin synthesis [37].

In the present study, postmenopausal endometrial sections did not express any immunoreactivity for either Flt1 or KDR. This was in accordance with the results of other investigators who found that the mean serum VEGF concentrations are lower in postmenopausal women than in premenopausal women [43].

In the current work, the functional relationship between uterine thickness and the expression of Flt1 and KDR was difficult to interpret, although it is expected that angiogenesis would increase when endometrial thickness increases. This was in accordance with the results of other investigators who observed intensified expression of Flt1 and KDR in the secretory phase, coinciding with an increase in uterine thickness [44].

It is also observed that angiogenesis is reduced with aging and this coincides with the decreased proliferative capacity in old age and upregulation of expression of natural endogenous inhibitors of angiogenesis during aging. Reduction in VEGF expression in old animals is at least in part due to a defect in transcriptional regulation. Aging itself enhances the sensitivity of endothelial cells toward apoptotic stimuli [45].

It was determined that the expression of VEGF and its receptors as well as that of vascular endothelial growth inhibitors (VEGIs) in the bovine uterus during the follicular and luteal phases varied in different cell types. This suggests that depending on the stage of the sexual cycle these factors may mediate the establishment of an appropriate environment for the nutritional supply and implantation of the embryo, primarily by stimulation of angiogenesis but also by increasing the secretory activity of the epithelial cells in the uterus. Furthermore, this indicates that ovarian steroid hormones play a significant role in regulating the expression of VEGF and its receptors as well as VEGI [46]. In the bitch uterus, cyclic changes may be precisely regulated by the combined functions of VEGF family members, angiogenic VEGF and VEGF-Rs, and by the angiogenesis inhibitor VEGI, which can help in cases of impaired angiogenesis, such as in those requiring *in-vitro* fertilization [47].

Conclusion

The upregulation of Flt1 and KDR could be involved in regulating endometrial endothelial cell proliferation and in increasing endometrial vascular permeability, especially at implantation sites.

Recommendations

Further studies on Flt1 and KDR are needed to help prolong the life span of endometrial cells aiming at maintaining a successful pregnancy, especially in women suffering from habitual early abortions or from repeated failure of implantation in '*in-vitro* fertilization programs'.

More experimental studies should be carried out to clear the correlation between hormonal replacement therapies and expression of VEGF-Rs and incidence of endometrial adenocarcinoma.

Acknowledgements

Conflicts of interest

There is no conflict of interest to declare.

References

1 Reynolds LP, Grazul-Bilska AT, Redmer DA. Angiogenesis in the corpus luteum. Endocrine 2000; 12:1–9.
2 Tran J, Master Z, Yu JL, Rak J, Dumont DJ, Kerbel RS. A role for survivin in chemoresistance of endothelial cells mediated by VEGF. Proc Natl Acad Sci USA 2002; 99:4349–4354.
3 Ferrara N, Gerber H-P. The role of vascular endothelial growth factor in angiogenesis. Acta Haematol 2001; 106:148–156.
4 Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu X-F, Breitman ML, Schuh AC. Failure of blood-island formation and vasculogenesis in Flk-1 deficient mice. Nature 1995; 376:62–66.
5 Fong G-H, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 1995; 376:66–70.
6 Matsumoto Y, Tanaka K, Hirata G, Hanada M, Matsuda S, Shuto T, Iwamoto Y. Possible involvement of the vascular endothelial growth factor-Flt-1-focal adhesion kinase pathway in chemotaxis and the cell proliferation of osteoclast precursor cells in arthritic joints. J Immunol 2002; 168:5824–5831.
7 Zeng H, Dvorak HF, Mukhopadhyay D. Vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF) receptor-1 down-modulates VPF/VEGF receptor-2-mediated endothelial cell proliferation, but not migration, through phosphatidylinositol 3-kinase-dependent pathways. J Biol Chem 2001; 276:26969–26979.
8 Yang S, Toy K, Ingle G, Zlot C, Williams PM, Fuh G, *et al.* Vascular endothelial growth factor-induced genes in human umbilical vein endothelial cells: relative roles of KDR and Flt-1 receptors. Arterioscler, Thromb Vasc Biol 2002; 22:1797–1803.
9 LeCouter J, Moritz DR, Li B, Phillips GL, Liang XH, Gerber H-P, *et al.* Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. Science 2003; 299:890–893.
10 Perrot-Applanat M. Hormonal regulation of vascular cell function: angiogenesis. In: Bikfalvi A, editor. *Encyclopedic reference of vascular biology and pathology*. 1st ed. Heidelberg: Springer Verlag; 2000. pp. 157–162.
11 Gluck JP, Di Pasquale T, Orlans B. *Applied ethics in animal research (philosophy, regulation, and laboratory applications)*. West Lafayette, IN, USA: Purdue University Press; 2002.
12 Kiernan JA. *Histological and histochemical methods: theory and practice*. 3rd ed. London, UK: Arnold publisher; 2001. pp. 111–162.
13 Bancroft JD, Cook HC. Immunocytochemistry. In: Bancroft JD Cook HC, Turner DR, editors. *Manual of histological techniques and their diagnostic applications*. 2nd ed. New York: Churchill Livingstone; 1994. pp. 263–325.
14 Armitage P, Berry G. Statistical methods. In: Armitage P, Berry G, editors. *Medical research*. 3rd ed. London, UK: Blackwell Scientific Publications; 1994. pp. 12–48.
15 Smith SK. Angiogenesis, vascular endothelial growth factor and the endometrium. Hum Reprod Update 1998; 4:509–519.
16 Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature 2000; 407:242–248.
17 Maharaj ASR, Saint-Geniez M, Maldonado AE, D'Amore PA. Vascular endothelial growth factor localization in the adult. Am J Pathol 2006; 168:639–648.
18 Saint-Geniez M, Maldonado AE, D'Amore PA. VEGF expression and receptor activation in the choroid during development and in the adult. Invest Ophthalmol Vis Sci 2006; 47:3135–3142.
19 Wood GA, Fata JE, Watson KLM, Khokha R. Circulating hormones and estrous stage predict cellular and stromal remodeling in murine uterus. Reproduction 2007; 133:1035–1044.
20 Naciff JM, Overmann GJ, Torontali SM, Carr GJ, Tiesman JP, Richardson BD, Daston GP. Gene expression profile induced by 17α-ethynyl estradiol in the prepubertal female reproductive system of the rat. Toxicol Sci 2003; 72:314–330.
21 Radi ZA, Khan NK. Comparative expression and distribution of c-fos, estrogen receptorα (ERα), and p38α in the uterus of rats, monkeys, and humans. Toxicol Pathol 2006; 34:327–335.
22 Burroughs KD, Fuchs-Young R, Davis B, Walker CL. Altered hormonal responsiveness of proliferation and apoptosis during myometrial maturation and the development of uterine leiomyomas in the rat. Biol Reprod 2000; 63:1322–1330.
23 Gebhardt S, Merkl M, Herbach N, Wanke R, Handler J, Bauersachs S. Exploration of global gene expression changes during the estrous cycle in equine endometrium. Biol Reprod 2012; 87: article no. 136:1-13
24 Marusak RA, Radi ZA, Obert L. Expression of Ki-67 in the uterus during various stages of the estrous cycle in rats. Exp Toxicol Pathol 2007; 59:151–155.
25 Westwood FR. The female rat reproductive cycle: a practical histological guide to staging. Toxicol Pathol 2008; 36:375–384.
26 Ma L, Yao M, Maas RL. Genetic control of uterine receptivity during implantation. Semin Reprod Endocrinol 1999; 17:205–216.
27 Tassell W, Slater M, Barden JA, Murphy CR. Endometrial cell death during early pregnancy in the rat. Histochem J 2000; 32:373–379.
28 Tawia SA, Rogers PAW. *In vivo* microscopy of the subepithelial capillary plexus of the endometrium of rats during embryo implantation. J Reprod Fertil 1992; 96:673–680.
29 Sivridis E, Giatromanolaki A. Proliferative activity in postmenopausal endometrium: the lurking potential for giving rise to an endometrial adenocarcinoma. J Clin Pathol 2004; 57:840–844.
30 Diel P. Tissue-specific estrogenic response and molecular mechanisms. Toxicol Lett 2002; 127:217–224.
31 Guangqi E, Cao Y, Bhattacharya S, Dutta S, Wang E, Mukhopadhyay D. Endogenous vascular endothelial growth factor-A (VEGF-A) maintains endothelial cell homeostasis by regulating VEGF receptor-2 transcription. J Biol Chem 2012; 287:3029–3041.
32 Sharkey AM, Day K, McPherson A, Malik S, Licence D, Smith SK, Charnock-Jones DS. Vascular endothelial growth factor expression in human endometrium is regulated by hypoxia. J Clin Endocrinol Metab 2000; 85:402–409.

33 Iruela-Arispe ML, Rodriguez-Manzaneque JC, Abu-Jawdeh G. Endometrial endothelial cells express estrogen and progesterone receptors and exhibit a tissue specific response to angiogenic growth factors. Microcirculation 1999; 6:127–140.

34 Zachary I, Gliki G. Signaling transduction mechanisms mediating biological actions of the vascular endothelial growth factor family. Cardiovasc Res 2001; 49:568–581.

35 Olsson A-K, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling – in control of vascular function. Nat Rev Mol Cell Biol 2006; 7:359–371.

36 Plaks V, Kalchenko V, Dekel N, Neeman M. MRI analysis of angiogenesis during mouse embryo implantation. Mag Reson Med 2006; 55:1013–1022.

37 Brownbill P, McKeeman GC, Brockelsby JC, Crocker IP, Sibley CP. Vasoactive and permeability effects of vascular endothelial growth factor-165 in the term *in vitro* dually perfused human placental lobule. Endocrinology 2007; 148:4734–4744.

38 Gambino LS, Wrefordm NG, Bertram JF, Dockery P, Lederman F, Rogers PAW. Angiogenesis occurs by vessel elongation in proliferative phase human endometrium. Hum Reprod 2002; 17:1199–1206.

39 Krikun G, Schatz F, Taylor R, Critchley HOD, Rogers PAW, Huang J, Lockwood CJ. Endometrial endothelial cell steroid receptor expression and steroid effects on gene expression. J Clin Endocrinol Metab 2005; 90:1812–1818.

40 Sherer DM, Abulafia O. Angiogenesis during implantation, and placental and early embryonic development. Placenta 2001; 22:1–13.

41 Silva LA, Klein C, Ealy AD, Sharp DC. Conceptus-mediated endometrial vascular changes during early pregnancy in mares: an anatomic, histomorphometric, and vascular endothelial growth factor receptor system immunolocalization and gene expression study. Reproduction 2011; 142:593–603.

42 Sugino N, Kashida S, Karube-Harada A, Takiguchi S, Kato H. Expression of vascular endothelial growth factor (VEGF) and its receptors in human endometrium throughout the menstrual cycle and in early pregnancy. Reproduction 2002; 123:379–387.

43 Hague S, Manek S, Oehler MK, MacKenzie IZ, Bicknell R, Rees MCP. Tamoxifen induction of angiogenic factor expression in endometrium. Br J Cancer 2002; 86:761–767.

44 Meduri G, Bausero P, Perrot-Applanat M. Expression of vascular endothelial growth factor receptors in the human endometrium: modulation during the menstrual cycle. Biol Reprod 2000; 62:439–447.

45 Hoffmann J, Haendeler J, Aicher A, Rössig L, Vasa M, Zeiher AM, Dimmeler S. Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli: important role of nitric oxide. Circ Res 2001; 89:709–715.

46 Sağsöz H, Saruhan BG. The expression of vascular endothelial growth factor and its receptors (flt1/fms, flk1/KDR, flt4) and vascular endothelial growth inhibitor in the bovine uterus during the sexual cycle and their correlation with serum sex steroids. Theriogenology 2011; 75:1720–1734.

47 Sağsöz H, Liman N, Küçükaslan T, Saruhan BG. Immunolocalization of vascular endothelial growth factor, its receptors (flt1/fms, flk1/KDR, flt4) and vascular endothelial growth inhibitor in the bitch uterus during the sexual cycle. Anim Reprod Sci 2013; 140:241–254.

الملخص العربى

دراسة نسيجية مناعية عن استظهار دلالات العامل المحفز لنمو النسيج المبطن للأوعية الدموية (إف- إل- تى 1 , كى دى آر) فى الغشاء المخاطى المبطن للرحم أثناء مراحل الدورة التناسلية لإناث الفئران

أمل مصطفى عباس، محمد صلاح الجندي[1]، نهى محمد عفيفى و أسماء محمد السيد علي[1]

قسم الهستولوجيا - كلية الطب - جامعة القاهرة وجامعة الفيوم[1]

المقدمة: تكوين الأوعية الدموية هي عملية مهمة في نمو بطانة الرحم وغرس الجنين، عامل النمو البطاني الوعائي ينظم هذه العملية من خلال التفاعل مع مستقبلاته الخاصة (إف- إل- تى 1، كى دى آر).

هدف الدراسة: تم تنفيذ هذا العمل لدراسة الصبغة المناعية لمستقبلات عامل نمو بطانة الاوعية في بطانة الرحم في مختلف الأعمار والمراحل الإنجابية وربط بينها وبين الصور النسيجية في هذه المراحل.

الأدوات و الطرق المستخدمة: تم إستخدام سبعون من الجرذان في هذه الدراسة: تم تقسيمها إلى سبع مجموعات، مجموعة واحدة في الفترة السابقة للبلوغ في عمر 4 - 6 أسابيع، وخمسة مجموعات في الفترة الإنجابية في سن 6 حتي 10 أشهر والتي تشمل الحوامل وآخر مجموعة فى فترة ما بعد انقطاع الطمث في سن ال 15 - 18 شهرا. وتم أخذ عينات من الرحم من الفئران و تجهيزها و صباغتها بالهيماتوكسلين و الإيوسين و كذلك صبغتها لمستقبلات عامل النمو البطاني الوعائي (إف- إل- تى 1، كى دى آر) مع تحليل النتائج باستخدام جهاز تحليل الصورة بالكمبيوتر لقياس متوسط سمك جدار الرحم وكذلك قياس شدة التفاعل المناعى ضد دلالات العامل (ڤى .إى . چى . إف) وتم التحليل الإحصائى الكمى للنتائج.

النتائج: التعبير عن شدة التفاعل المناعى ضد دلالات عامل النمو البطاني الوعائي كانت أعلى شدة في المجموعة العمرية البالغة خاصة في مرحلة مقدمات الودق تليها مرحلة الشبق وهذا يدعم دور الهرمونات الجنسية خاصة هرمون الاستروجين في تنظيم مستقبلات عامل النمو البطاني الوعائي (إف- إل- تى 1، كى دى آر) و قد كانت مستقبلات (إف إل تى 1) هى السائدة في خلايا بطانة الرحم و النسيج الحشوى وكذلك الخلايا الجنينية بينما كانت مستقبلات (كى دى آر) هى السائدة في خلايا بطانة الأوعية الدموية فى الرحم.

كان هناك تفاوتاً كبيراً إحصائيا بين المجموعات المختلفة.

الخلاصة: يمكن أن تشترك مستقبلات عامل النمو البطاني الوعائي (إف- إل- تى 1، كى دى آر) فى تنظيم تكاثر الخلايا البطانية فى الرحم وزيادة نفاذية الأوعية الدموية وخاصة على مواقع التوطين الجنينية.