# Localization of connective tissue growth factor synchronizing with histological changes in the mouse uterus during the estrus cycle

**Abdel-Rahman S. Sultan[1], Yasmin M.M. Ismaiel[*2], Ahmed S. A. Harabawy[2], Ahmed Th. A. Ibrahim[2]**

[1] Zoology Department, Faculty of Science, Al-Azhar University, Assiut, Egypt

[2] Department of Zoology, Faculty of Science, New Valley University, Al-Kharga, New Valley, 72511 Egypt.

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## Abstract

The mouse uterus undergoes continual histological and immunohistochemical changes during the estrous cycle; these changes are regulated by the levels of ovarian hormones. Connective tissue growth factor (CTGF), one of the first CCN members, is a multifunctional signaling modulator participating in a wide variety of physiological and pathological processes, such as angiogenesis, osteogenesis, renal disorders, and carcinogenesis. By using histological and immunohistochemical investigations, this study is to focus on the changes of the expression levels of CTGF which are synchronized with the histological changes of the mouse endometrium during the estrous cycle. At proestrus, the luminal epithelium is columnar, the uterine glands are many and distributed evenly throughout the mucosa, at estrus, the luminal epithelial cells are columnar and the lumina of uterine glands are wide pointing to functional activity. Metestrus showed degeneration of endometrial columnar epithelium cells and endometrial atrophy continued degeneration of endometrial epithelial cells. At the diestrus, luminal epithelium is low columnar and has no basement membrane. Moreover, CTGF expressed strongly in the stroma of the endometrium, the connective tissue and blood vessels of myometrium and perimetrium at proestrus and estrus phases. At metestrus, the expression of CTGF was reduced to extremely low level in the endometrial stromal cells. In contrast, CTGF was expressed moderately in the perimetrium, and in endometrial blood vessels and the connective tissues of myometrium. At the diestrus, CTGF was expressed strongly in the blood vessels of endometrium and myometrium and expressed moderately in the stromal cells of endometrium. The luminal epithelium and uterine glands showed weak signal. Taking together, our results suggested that the growth of the endometrial stroma and blood vessels is associated with the strong expression level of CTGF during the proestrus and estrus phases.

**Keywords:** Estrous cycle, connective tissue growth factor (CTGF), proestrus, estrus, metestrus.

## Introduction

The female reproductive system of the mouse undergoes spectacular cyclic morphological, histochemical, biochemical changes during the estrus cycle in response to hormonal stimuli [1]. Estrogen secretion increases from a low during metestrus and diestrus to a peak of in proestrus [2] and progesterone secretion increases in estrus [3, 4].

Determining the estrus stage is necessary for choosing the female mouse suitable for mating with a male, in order to accomplish a timely pregnancy. In the mouse, there are four phases comprise the estrous cycle, (proestrus, estrus, metestrus, and diestrus) and lasts for 4 to 5 days [5]. The wall of the mouse uterus consists of three layers: (1) a luminal mucous membrane lining (endometrium), (2) an intermediate smooth muscle layer (myometrium), and (3) an outer serosa, connective tissue and mesothelium (perimetrium). The endometrium is the layer in which implantation takes place. The endometrial tissue's distinctive feature is that, throughout pregnancy, it responds to cyclical hormonal cues from the ovaries and the implanting embryo by changing morphologically and molecularly. during the estrous cycle, the endometrial tissues undergo remarkable periodic morphological changes, growth, degeneration of the epithelial cells [6], waves of stromal cell proliferation, differentiation, apoptosis, and tissue breakdown by metalloproteases, and ultimate regeneration [7].

During endometrial changes, the endometrial blood vessels continuously undergo dramatic and regular regeneration, which involves angiogenesis and neovascularization [8]. Under the hormonal control these histological changes are considered to be regulated by cytokines and growth factors, several of which are members of the transforming growth factor-β (TGF-β) superfamily [9]. The bone morphogenetic protein (BMP) family is the largest subgroup of ligands in the TGF-β superfamily, which is closely associated with biological processes and cellular events involved in uterine cell proliferation, differentiation, apoptosis,



* Corresponding authors: y.mahrous@sci.nvu.edu.eg

and tissue remodeling [9]. In the bitch uterus, the cyclic changes may be precisely regulated by the combined functions of vascular endothelial growth factor VEGF family members, angiogenic VEGF and VEGF receptors, and the angiogenesis inhibitor VEGI [10].

Connective tissue growth factor (CTGF), one of the first CCN members, is a multifunctional signaling modulator participating in a wide variety of physiological or pathological processes, such as angiogenesis, osteogenesis, renal disorders, and carcinogenesis [11]. The acronym CCN was conceptualized after the initials of the first three proteins discovered in this family, namely: Cysteine rich angiogenic inducer 61 (CYR61), connective tissue growth factor (CTGF) and Nephroblastoma overexpressed protein (NOV) [12]. Moreover, CTGF was localized at high levels to both luminal and glandular uterine epithelial cells during the diestrous and early proestrous stages and at much lower levels in the stroma or myometrium. Epithelial expression of CTGF was considerably reduced at estrus. Complex hormonal, growth factor and complementary receptor interactions also appear to play important roles in various events (proliferation, differentiation) in normal cyclic uterine tissues [13]. Insulin- like growth factor-1 (IGF-1) and epidermal growth factor (EGF) have been associated with uterine growth [14] EGF appears to be a potent mitogen in mouse uterine cell cultures [15], and EGF-specific antibody blocks estrogen- induced uterine growth [16]. The objective of the present study is to focus on the changes of the expression levels of CTGF which are synchronized with the histological changes of the mouse endometrium during the estrous cycle.

## Materials and methods

### Animals and experimental design

The Ethics Committee for Animal Experimentation of the Faculty of Science, Al-Azhar University, Assiut approved the experiment. Adult virgin female mice (22-24 g) at an age of 8-10 weeks were used. The habitat for Swiss mice during the experiment is maintained under stringent regulation at the Faculty of Science animal facility, Al-Azhar University, Assiut with the accessibility of sufficient meals and water. The female mice (n = 20) were divided into four groups; (1) pro estrus group (n = 5), (2) estrus group (n = 5), (3) metestrus group (n = 5), and (4) diestrus group (n = 5). The animals were euthanized by cervical dislocation.

### The visual assessment

The visual assessment of the vaginal opening was performed during the estrus phases and photographed by Oppo mobile phone camera.

### Vaginal smear/cytology

Vaginal cytology is also commonly accepted, just like the ocular evaluation. It appears to be the method most frequently employed to identify the stages of the estrous cycle. It is reasonably priced and minimally invasive. This technique is accurate and dependable even if it demands a certain level of proficiency to examine the vaginal discharge cells under a microscope. However, It has also been noted that this approach is time-consuming and laborious [17]. The animal and its forepaws are restrained while being evaluated. To see the vagina, the tail is raised. A small amount (100 µl) of Phosphate-buffered saline is carefully introduced to cleanse the vaginal cells. Draw the liquid back into the tip after releasing it gradually into the vagina; this should be repeated about 4 to 5 times in the same sterile latex bulb. After that, the fluid with a few drops of cell suspension is put onto a glass slide, air-dried and stained by Giemsa stain. The slides were immediately checked under a light microscope and captured by OLYMPUS PEN Lite E-PL7 camera.

### Morphological examination of the mouse uterus

The animals were euthanized by cervical dislocation during the estrus phases and the uterus was removed and examined and photographed by Oppo mobile phone camera.

**Histological studies**

Uterine horns were dehydrated in 100% ethyl alcohol for one hour, fixation by Carnoy's fluid for 2 hours, then cleared in xylene twice for one hour each, then placed in paraffin wax and baked twice for one hour each at 60 °C. Blocks of paraffin wax were sectioned using the Leica RM microtome to a 7 µm thickness, and the sections were then put on glass slides, Rehydrated with descending series of ethyl alcohol and distilled water, after being dewaxed by xylene, then stained with Hematoxylin and Eosin (H & E), then dehydrated using a sequence of increasing ethyl alcohol concentrations (50, 70%, 90%, and 100%), followed by xylene clearance and DPX mounting [18]. Photographs of stained slides were taken using an OLYMPUS PEN Lite E-PL7 camera.

**Immunohistochemical studies:**

Immunohistochemistry's basic idea is to use enzymatic reactions to visualize target antigens through antigen-antibody interactions. Tissue preparations embedded in paraffin were subjected to immunohistochemical analyses and by using the EconoTek Kit (code: 800-729-8350, EconoTek Kit, ScyTek Laboratories company, Logan, USA) and Horseradish peroxidase (HRP) method. The preparations were rinsed twice for 5 minutes in 0.1 M phosphate buffer saline (PBS). The sections were incubated for 10 minutes at room temperature in a protein blocking solution (EconoTek Super block) to avoid nonspecific staining. The preparations were then incubated for one hour at room temperature with the primary antibody (Anti-CTGF Rabbit polyclonal IgG, Spring Bioscience, Canada, 1:200 dilution in Phosphate buffer). Following two 5-minute washes in 0.1 M PBS, the preparations were incubated for 30 minutes at room temperature with biotinylated secondary antibodies (EconoTek Biotinylated Anti-Polyvalent) before being rinsed twice for five minutes in 0.1 M PBS. Following a 30-minute incubation period in HRP conjugate, the preparations were washed twice for a total of five minutes in 0.1 M PBS. The formulations were exposed to 303-diaminobenzidine hydrochloride (DAB) for 5–15 minutes to visualize the reaction. The sections were dehydrated and cleared by xylene [19]. After DPX mounted the slides, the OLYMPUS PEN Lite E-PL7 camera was used to view and take pictures of the sections.

## Results

**The visual assessment**

The vaginal entrance is big, swollen, and wet during the proestrus period. The vulva's dorsal and ventral lips have longitudinal folds, and the tissues are reddish-pink (Fig. 1 A). The vaginal aperture is greater during estrous than during proestrus, but the smaller external appearance due to strong swelling of the vulva, the vulva's dorsal and ventral lips have more noticeable longitudinal folds, and the tissues are less wet and lighter pink (Fig. 1 B). the vaginal aperture is dry and pallid during metestrus, and the vulva does not enlarge, there are white cellular debris (Fig. 1 C). one of the characteristics of diestrus is an extremely wet vaginal opening, which is very small with no tissue swelling (Fig. 1 D).

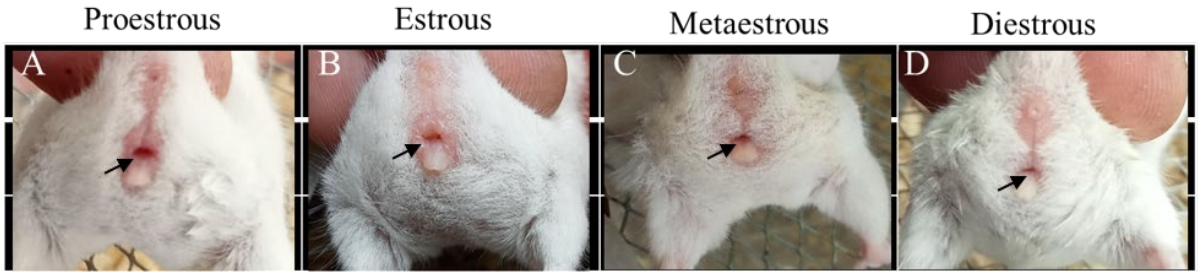


**Fig. 1 photographs of the vaginal opening (arrows) of the mouse showing different phases of estrous cycle. A-Proestrus, B Estrus, C- Metestrus, D- Diestrus.**

**Vaginal smear/cytology**

The types of cells and their proportional numbers in vaginal smears are used to categorize the stages of the estrous cycle. three kinds of cells have been detected, leucocytes, cornified epithelial cells and nucleated epithelial cells. Many rounded nucleated cells can be seen separately or in clusters during the proestrus phase, also few non-nucleated cornified epithelial cells. No leucocytes were detected (Fig. 2 A). The estrus phase illustrates lots of irregular non-nucleated cornified epithelial cells and occasionally, nucleated epithelial cells. No leucocytes were detected (Fig. 2 B). At metestrus, a lot of leucocytes and non-nucleated cornified epithelial cells have been detected (Fig. 2 C). Diestrus shows a lot of polymorphonuclear leukocytes and a few nucleated epithelial cells (Fig. 2 D). Morphologically, the widths of the mouse uterus at proestrus and estrus are bigger than that at metestrus and diestrus (Fig. 3 A, B, C and D).

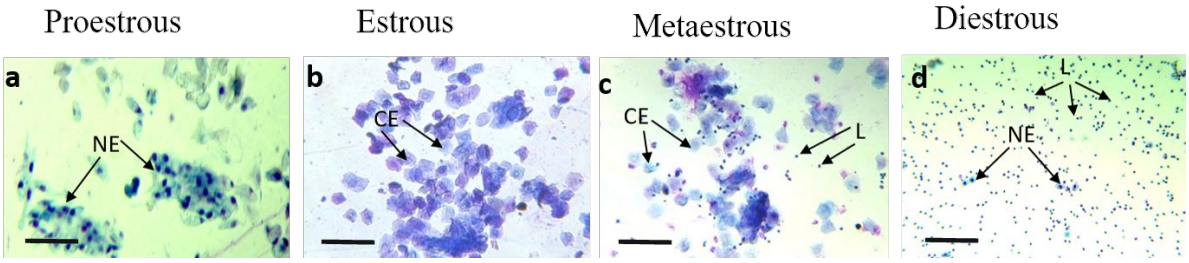


**Fig. 2 Microscopic Photos of vaginal smear of the mouse showing different phases of estrous cycle according to cell types. a-Proestrus, b-Estrus, c-Metestrus, d- Diestrus.**
The types of cells include cornified epithelial cells (CE), leucocytes (L) and nucleated epithelial cells (NE). The slides were stained with Giemsa stain and examined under a light microscope immediately. Scale par; (A, B, C, D) 2.5µm.

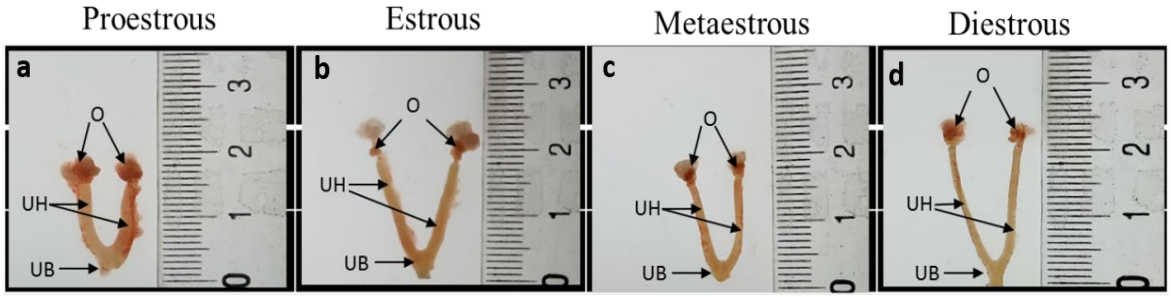


**Fig. 3 photographs of the mouse uterus in the different phases of estrous cycle. a-Proestrus, b-Estrus, c- Metestrus, d- Diestrus.** Ovary (O), uterine horn (UH), uterine body (UB).

**Histological changes of the mouse uterus during the estrus cycle**

At proestrus, the luminal epithelium is columnar, and mitosis of epithelial cells is seen with minimal inflammatory cell infiltration and no degeneration (Fig. 4 D). The uterine glands are enlarged, and the outlines of their cells are visible. The lack of mitoses suggests that the glands are not growing very much during hyperfunction (Fig. 4 E). There are many glands that are uniformly spaced across the mucosa, Cellular proliferation was seen in the thicker stromal connective tissue, and there were more blood vessels (Fig. 4 A&B). The proestrus shows an undulating endometrium with projection in to the lumen (Fig. 4 A, B & C). At estrus, Columnar luminal epithelial cells undergo somewhat frequent mitoses, and the epithelium is devoid of leucocytes, The epithelium's basement membrane is distinct and substantial (Fig. 4 I). The uterine glands' broad lumina indicate functional activity (Fig. 4 J).

During Metestrus, endometrial columnar epithelium cells began to degenerate, and endometrial atrophy further accelerated this process. The uterine epithelium's basement membrane is completely absent (Fig. 4 K, L, M, N and O). At diestrus, A tiny, avascular lumen that resembles a slit is present, the luminal epithelium is low columnar and has no basement membrane. At this stage, there is stromal oedema and the endometrium is extremely thin with atrophied endometrial matrix (Fig. 4 P, Q, R, S, and T).

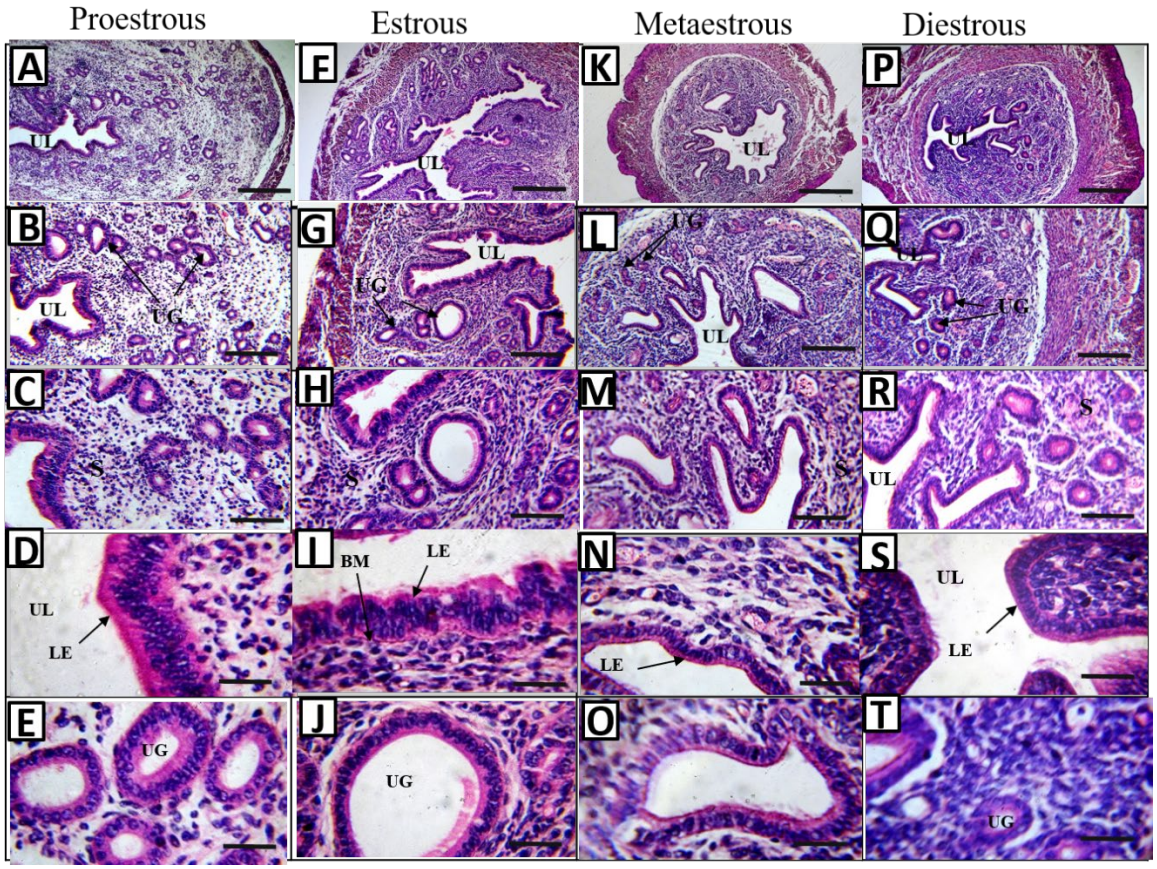


**Fig. 4. Microscopic photos of cross sections of the mice uteri during different phases of estrus cycle.** Histology of the uterine horn of proestrus phase (A, B, C, D and E), estrus phase (F, G, H, I and J), metestrus phase (K, L, M, N and O), and diestrus phase (P, Q, R, S, and T). (H & E staining). Scale bar: (A, F, K, and P) 50µm, (B, G, L and Q) 25µm, (C, H, M and R)10µm, (D, E, I, J, N, O, S and T) 5µm. luminal epithelium (LE), stroma (S), uterine gland (UG), uterine lumen (UL).

**Immunohistochemical localization of CTGF of the mouse uterus during the estrus cycle**

At proestrus, the expression of (CTGF) was localized strongly in the stroma of the endometrium, the connective tissue and blood vessels of myometrium and perimetrium. The luminal and glandular epithelial cells showed weak signal (Fig. 5 A, B, C, D and E). At estrus phase, the expression of (CTGF) was similar to that at proestrus localized strongly mainly in the stromal cells of endometrium and in the myometrium particularly, the connective tissue and blood vessels (Fig. 5 F, G, H, I and J). At metestrus, the expression of CTGF was reduced to very low level in the endometrial stromal cells. In contrast, CTGF was expressed moderately in the perimetrium, and in endometrial blood vessels and the connective tissues of myometrium (Fig. 5 K, L, M, N and O). At diestrus, the CTGF was expressed strongly in the blood vessels of endometrium and myometrium, and expressed moderately in the stromal cells of endometrium. The luminal epithelium and uterine glands showed weak signal (Fig. 5 P, Q, R, S, and T).

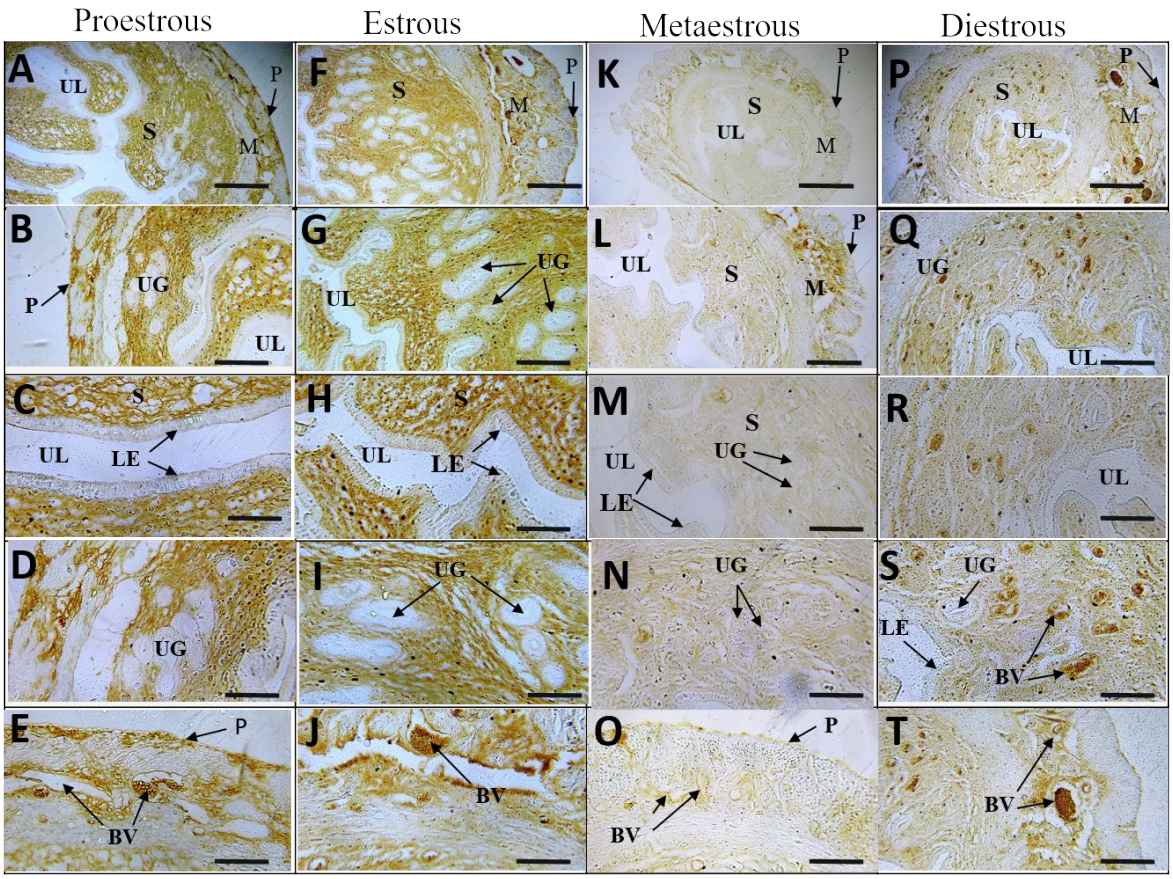


**Fig. 5. Microscopic photos of cross sections of the mice uteri during different phases of estrus cycle.** Immunohistochemical localization of CTGF of the uterine horn at proestrus phase (A, B, C, D and E), estrus phase (F, G, H, I and J), metestrus phase (K, L, M, N and O), and diestrus phase (P, Q, R, S, and T). Scale bar: (A, F, K, and P) 50µm, (B, G, L and Q) 25µm, (C, D, E, H, I, J, M, N, O, R, S and T) 10µm. blood vessels (BV), luminal epithelium (LE), myometrium (M), perimetrium (P), stroma (S), uterine gland (UG), uterine lumen (UL).

## Discussion

During estrous cycle, the stages were determined by observing different morphological and histological changes in the reproductive tract of cycling females, as well as behavioral changes (such as accepting copulation), These included the microscopic alterations in the ovaries and uterine tissue as well as the macroscopic alterations in the vulva (like swelling), vaginal excretions (like bleeding & mucus), and uterus (like congestion) [20]. Both rats and mice often have 4-5 day estrus cycles, while some individuals may occasionally have 6-day cycles [21, 22]. A detailed macroscopical and microscopical description of the vaginal canal alterations that take place during the estrus cycle was provided by histology and cytology. Additionally, the alterations that take place in other reproductive organs throughout the estrus cycle were linked to these vaginal modifications in the rat and mouse [23].

The current study revealed that Estrous cycle phases can be ascertained using a variety of methods. Choosing the right approach to use requires careful consideration. In live animals, cytology of the vagina continues to be the perfect standard, on which other techniques are validated [24]. The most important target organs for progesterone and estrogen, whose levels fluctuate often throughout the estrus cycle, include the uterus, cervix, and vagina [25]. Studies have shown the high expression of estrogen and progesterone receptors in endometrial tissue [26]. The vaginal smears of mature female mice were

analyzed under a microscope in this study, and the results showed a high degree of consistency with the cytology patterns used to describe the various stages of the experimental animals' estrous cycles [27]. Leukocytes, cornified epithelial cells, and nucleated epithelial cells are the main types of cells seen in vaginal smears. The percentage of two cell types seen in the vaginal smear is used to determine the estrous stage: (1) the epithelial cells which are originate from the stratified squamous epithelium lining the vagina and (2) polymorphonuclear leukocytes, primarily neutrophils [28]. The proportion of cornified epithelial cells, nucleated epithelial cells, and leukocytes was used to characterize each stage. Nucleated epithelial cells predominate in the proestrus stage of the estrus cycle, while cornified epithelial cells are very abundant in the estrus stage. mixture of all three cell types characterizing the metestrus stage, with leukocytes making up the largest proportion. Leukocyte invasion starts during metestrus and continues into diestrus [29].

Our results showed that the mouse uterus undergoes some histological changes during the estrus cycle, these alterations comprised the endometrial columnar epithelium's recurrent growth and exfoliation as well as the glands' recurring modifications. At proestrus, the columnar luminal epithelium showed the mitoses, the uterine glands are many and distributed evenly throughout the mucosa. Cellular growth was seen in the thickened stromal connective tissue, and there were more blood vessels overall. At estrus, the luminal epithelial cells are columnar, mitosis is moderately frequent. The basement membrane of the epithelium is distinct and heavy. The lumina of uterine glands are wide pointing to functional activity. Metestrus showed Endometrial atrophy and endometrial columnar epithelium cell degeneration. At diestrus, the luminal epithelium is low columnar and the endometrium is very thin with endometrial matrix atrophy, this change coincides with Ajayi & Akhigbe in their study on rats. The ovarian hormones are responsible for these changes occurring during the estrous cycle [28].To understand the mechanism by which these changes are regulated by ovarian hormones, Throughout the estrous cycle, the uterus changes morphologically in response to the steroidal environment created by the luteal and preovulatory processes [30].

Histological alterations in the luminal and glandular epithelium, stromal cells, uterine gland secretory activity, and the distention and shape of the uterine lumen are among the changes observed in the uterus, total uterine thickness with direct relationship of E2 levels, and inversely with P4 [28].

The present study showed that expression levels of CTGF during the estrus stages and found that (CTGF) has been expressed strongly in the stroma of the endometrium, the connective tissue and blood vessels of myometrium and perimetrium at proestrus and estrus phases. At metestrus, the expression of CTGF was reduced to very low level in the endometrial stromal cells. Moreover, CTGF was expressed moderately in the perimetrium, and in endometrial blood vessels and the connective tissues of myometrium. At diestrus, the CTGF has been expressed strongly in the blood vessels of endometrium and myometrium, and expressed moderately in the stromal cells of endometrium. The luminal epithelium and uterine glands showed weak signal. It has been suggested that a number of growth factors function in an autocrine or paracrine manner to mediate the estrogen-induced proliferation of uterine tissues [31]. Taken all together, we concluded that, the growth of the endometrial stroma and blood vessels is highly associated with the strong expression level of CTGF during the proestrus and estrus phases.

## References

1. Ramírez-González, J.A., et al., *Overview of the female reproductive system.* Exercise and human reproduction: induced fertility disorders and possible therapies, 2016: p. 19-46.
2. Bridges, G., et al., *Influence of the length of proestrus on fertility and endocrine function in female cattle.* Animal reproduction science, 2010. **117**(3-4): p. 208-215.
3. Norris, D.O. and J.A. Carr, *Vertebrate endocrinology*. 2020: Academic Press.

4. Guttenberg, I., *Plasma levels of "free" progestin during the estrous cycle in the mouse.* Endocrinology, 1961. **68**(6): p. 1006-1009.
5. Byers, S.L., et al., *Mouse estrous cycle identification tool and images.* PloS one, 2012. **7**(4): p. e35538.
6. Bai, X., et al., *Research progress of endometrial receptivity in patients with polycystic ovary syndrome: a systematic review.* Reproductive biology and endocrinology, 2021. **19**(1): p. 122.
7. Sağsöz, H., et al., *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.* Animal reproduction science, 2013. **140**(3-4): p. 241-254.
8. Chennazhi, K.P. and N.R. Nayak. *Regulation of angiogenesis in the primate endometrium: vascular endothelial growth factor*. in *Seminars in reproductive medicine*. 2009. © Thieme Medical Publishers.
9. Li, Y., et al., *Expression of bone morphogenetic protein 2, 4, and related components of the BMP signaling pathway in the mouse uterus during the estrous cycle.* Journal of Zhejiang University-SCIENCE B, 2014. **15**: p. 601-610.
10. Praderio, R.G., et al., *Vascular endothelial growth factor (VEGF) expression in histological endometritis in the bitch: An immunohistochemical study.* Reprod Domest Anim, 2022. **57**(9): p. 1088-1092.
11. Arnott, J.A., et al., *The role of connective tissue growth factor (CTGF/CCN2) in skeletogenesis.* Crit Rev Eukaryot Gene Expr, 2011. **21**(1): p. 43-69.
12. Holbourn, K.P., K.R. Acharya, and B. Perbal, *The CCN family of proteins: structure–function relationships.* Trends in biochemical sciences, 2008. **33**(10): p. 461-473.
13. Guzeloglu-Kayisli, O., U.A. Kayisli, and H.S. Taylor. *The role of growth factors and cytokines during implantation: endocrine and paracrine interactions*. in *Seminars in reproductive medicine*. 2009. © Thieme Medical Publishers.
14. Murphy, L.J. and A. Ghahary, *Uterine insulin-like growth factor-1: regulation of expression and its role in estrogen-induced uterine proliferation.* Endocrine reviews, 1990. **11**(3): p. 443-453.
15. Lim, H.J. and S. Dey, *HB-EGF: a unique mediator of embryo-uterine interactions during implantation.* Experimental cell research, 2009. **315**(4): p. 619-626.
16. Klein, R., S. Stiller, and I. Gashaw, *Epidermal growth factor upregulates endometrial CYR61 expression via activation of the JAK2/STAT3 pathway.* Reproduction, Fertility and Development, 2012. **24**(3): p. 482-489.
17. Ajayi, A.F. and R.E. Akhigbe, *Staging of the estrous cycle and induction of estrus in experimental rodents: an update.* Fertil Res Pract, 2020. **6**: p. 5.
18. Drury, R. and E. Wellington, *Carlton's Histo-pathological Techniques: Oxford University press*. 1976, Oxford, London, New York.
19. Buchwalow, I.B. and W. Böcker, *Immunohistochemistry: basics and methods*. 2010: Springer Science & Business Media.
20. Cora, M.C., L. Kooistra, and G. Travlos, *Vaginal cytology of the laboratory rat and mouse: review and criteria for the staging of the estrous cycle using stained vaginal smears.* Toxicologic pathology, 2015. **43**(6): p. 776-793.
21. Goldman, J.M., A.S. Murr, and R.L. Cooper, *The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies.* Birth Defects Research Part B: Developmental and Reproductive Toxicology, 2007. **80**(2): p. 84-97.
22. Holalagoudar, S., et al., *Rodent Estrous Cycle Pattern: Harmonizing the Cycle Evaluation and Interpretation.* Regulatory Toxicology and Pharmacology, 2024: p. 105768.

23. Arnon, G., L. Po-Ching, and K. CheMyong, *Vaginal fold histology reduces the variability introduced by vaginal exfoliative cytology in the classification of mouse estrous cycle stages.* Toxicologic pathology, 2014. **42**(8): p. 1212.
24. Ajayi, A.F. and R.E. Akhigbe, *Staging of the estrous cycle and induction of estrus in experimental rodents: an update.* Fertility research and practice, 2020. **6**: p. 1-15.
25. Yip, K.S., et al., *Changes in mouse uterine transcriptome in estrus and proestrus.* Biology of reproduction, 2013. **89**(1): p. 13, 1-12.
26. Potje, S.R., et al., *The effects of female sexual hormones on the endothelial glycocalyx.* Current Topics in Membranes, 2023. **91**: p. 89-137.
27. Cora, M.C., L. Kooistra, and G. Travlos, *Vaginal Cytology of the Laboratory Rat and Mouse: Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears.* Toxicol Pathol, 2015. **43**(6): p. 776-93.
28. Bertolin, K. and B.D. Murphy, *Reproductive tract changes during the mouse estrous cycle*, in *The guide to investigation of mouse pregnancy*. 2014, Elsevier. p. 85-94.
29. Cora, M., V. Sutherland, and G. Roberts, *Fetal Examinations and Vaginal Cytology*, in *Specifications for the Conduct of Toxicity Studies by the Division of Translational Toxicology at the National Institute of Environmental Health Sciences [Internet]*. 2023, National Institute of Environmental Health Sciences.
30. Das, P.K., J. Mukherjee, and D. Banerjee, *Female reproductive physiology*, in *Textbook of veterinary physiology*. 2023, Springer. p. 513-568.
31. Shiraga, M., et al., *Epidermal growth factor stimulates proliferation of mouse uterine epithelial cells in primary culture.* Zoological science, 2000. **17**(5): p. 661-666.