Experimental intravaginal and intrauterine endometritis model: which model is more useful?

ABSTRACT

This study aims to compare the newly created intravaginal endometritis model (IVM) with the intrauterine endometritis model (IUM). *E. coli* infusion was used as intravaginally for IVM and intrauterinally for IUM model. The animals were exeuted on the 7th day. Histopathological and biochemical analyses [malondialdehyde (MDA), glutathione (GSH), Endocan, Endoglin] were performed. A significant inflammation was determined in IVM and IUM compared to the control. A significant decrease in GSH and a significant increase in MDA and Endoglin were determined in IVM and IUM compared to the control. There was a statistically significant increase in the IUM and a numerical increase in the IVM compared to the control. Endometritis was determined by histopathological and biochemical analyses in both IUM and IVM model. It is suggested that intravaginal administration, which is easier to perform, can be used in experimental endometritis model studies.

Keywords: Endometritis, GSH, MDA, Endocan, Endoglin

INTRODUCTION

Endometritis is a widespread gynecological disease characterized by inflammation of endometrial glandular and stromal tissues (Demirel et al., 2019) with mainly resulting in infertility in human and animals. Pelvic inflammatory disease is a clinical infection that results in inflammation of the upper female reproductive tract, including the tube, ovaries, and uterus. Anaerobic gram-negative bacteria generally cause infection. The most common etiological agents are *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, and *Staphylococcus aureus* (Cohen et al., 2002; Ness & Brooks-nelson, 2000; Wiesenfeld & Paavonen, 2010). Endometritis not only causes infertility in farm animals but also causes serious economic losses by affecting milk yield and reproductive performance (Adnane et al., 2017; Demirel et al., 2019; Salah & Yimer, 2017; Sheldon & Dobson, 2004) whereas infertility is the ultimate consequence of the disease in humans (Demirel et al., 2019). Therefore, affecting fertility is important for both human and animal health. Inflammation in the endometrium may cause infertility by preventing the formation of a new cycle. It can also cause symptoms systemically due to its microbial causes (Salah & Yimer, 2017).

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Research Article

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The bacterial endotoxin-induced uterine infection affects fertility both locally and systemically in the postpartum period. The inflammation causes disruptions in the production of prostaglandin (PG) F2α and PGE2 in the endometrium. However, a change occurs in immune system mediators and cytokine production that may affect the function of the hypothalamus and pituitary (Herath et al., 2009; Sheldon et al., 2009). Detection of bacterial endotoxin lipopolysaccharide in follicular fluid is an indicator that uterine infection adversely affects follicular growth and function (Sheldon et al., 2002). In addition, uterine infection in farm animals causes prolongation of luteal activity and a longer interpregnancy interval (Mohammed et al., 2019).

Physiological reactive oxygen species (ROS) plays an important role in regulating many reproductive processes such as folliculogenesis, oocyte maturation, implantation, embryogenesis, and fetal-placental development. Endometriosis is associated with chronic inflammation and ROS are proinflammatory mediators that modulate cell proliferation (Ngô et al., 2009). As is known, oxidative stress during the formation of endometritis or genital tract inflammation causes pathophysiological disturbance and imbalance in the production of ROS. Therefore, ROS can directly or indirectly damage macromolecules in cases of inflammation (Demirel et al., 2019; Kuru et al., 2018).

Endoglin (CD105) is a transmembrane glycoprotein strongly expressed in several cell types such as macrophages, fibroblasts, and syncytiotrophoblasts, especially vascular endothelial cells (Mitsui et al., 2013). Endoglin dissolved in the plasma is found in the systemic circulation. Circulating endoglin and placental endoglin expression have been reported to be higher in patients with preeclampsia compared to normal pregnancies (Hawinkels et al., 2010). Endoglin expression is known to occur in many types of cancer such as breast, colorectal, and prostate cancers. In addition, increased circulating endoglin levels in colorectal, breast, and other tumors have been reported in studies. It is also known to increase in uterine infections (Mitsui et al., 2013).

Endocan (endothelial cell-specific molecule-1, ESM-1) is a chondroitin/dermatan sulfate proteoglycan specific to vascular endothelial cells that plays a very important role in angiogenesis and inflammation. Serum endocan levels have also been reported to increase in many tumor formations (Laloglu et al., 2017). Serum endocan concentration increases in women with endometriosis and can predict endometriosis with 93% sensitivity and 61% specificity (Güralp et al., 2020).

Experimental studies on endometritis are frequently conducted in recent years. Intrauterine applications are used in the endometritis rat model and this application method may cause some complications due to the need for surgical intervention. This study aims to compare biochemical and histopathological changes in models of intravaginal or intrauterine *Escherichia coli*-induced rat endometritis.

MATERIAL and METHOD

A 15-day period was given to all rats for the adaptation. The cycle periods of all rats were determined by vaginal cytology before starting the study (Cora et al., 2015) and the rats determined to be in the diestrus period were included in the study groups. Serum progesterone concentration of rats found in diestrus on vaginal cytology was also measured using a commercial kit on the AutoAnalyzer (Roche Cobas C501).

Blood was taken from the tail vein of the rats and centrifuged at 3000 RPM for 5 minutes and serum progesterone concentration was measured on the same day from the obtained sera. Rats with a progesterone level higher than

1 ng/mL were used in the study according to the results.

The study was conducted on 21 albino rats. The rats were divided into three groups, each group containing 7 rats, and they were fed as *ad-libitum* with standard normal rat pellet feed and drinking water for 11 days.

Control: Progesterone (P4) (Progestan®, Koçak Farma) was administered subcutaneously to rats at a daily dose of 16 mg/kg between the 0^{th} and 4^{th} days of the study.

Intravaginal endometritis model (IVM): P4 was administered subcutaneously to rats at a daily dose of 16 mg/kg between the 0^{th} and 4^{th} days of the study. *E. coli* was administered intravaginally at 25 µL and 1×10^5 CFU/rat on the 3^{rd} day (Figure 1).

Intrauterine endometritis model (IUM): P4 was administered subcutaneously to rats at a daily dose of 16 mg/kg between the 0^{th} and 4^{th} days of the study. *E. coli* was administered intrauterinally at 25 µL and 1×10^5 CFU/rat on the 3^{rd} day (Figure 1).

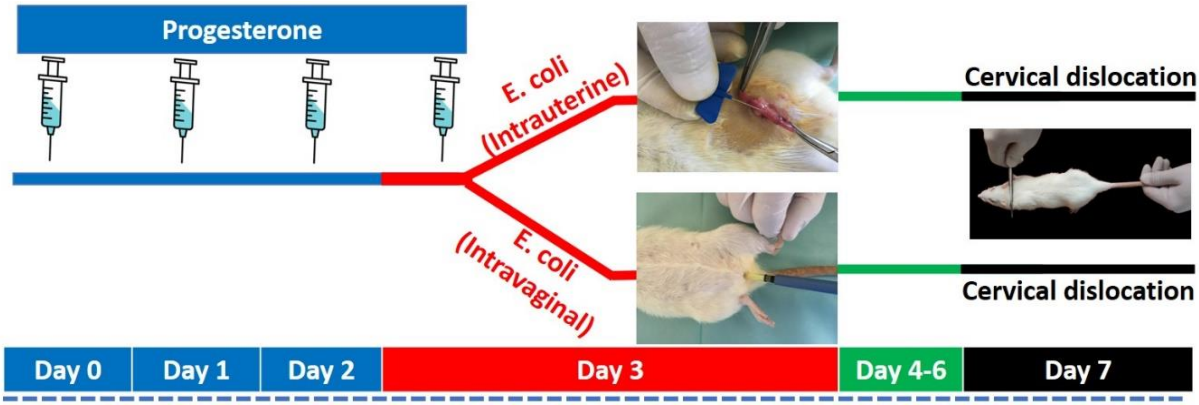

Figure 1. Intravaginal and intrauterine endometritis model protocol

Rats were euthanased at the end of the study (7 days after *E. coli* administration) under anesthesia [ketamine hydrochloride (75 mg/kg) (Ketalar®, Pfizer), and xylazine (15 mg/kg) (Rompun®, Bayer) intramuscular] by cervical dislocation method in accordance with ethical rules. Blood and tissue samples from rats were then taken for biochemical and histopathological analyses. Tissue samples taken for histopathological analysis were placed in 10% formaldehyde. Blood samples were centrifuged at 3000 RPM for 5 minutes and separated. Serum samples were stored at -80°C until the day of the analyses.

Preparation of E. coli strain

Escherichia coli ATCC 25922 strain was used in the study. The concentration of the bacteria was determined according to McFarland standards. McFarland 0.5 contains $1.5x10^8$ bacteria/ml (Garcia, 2010). The McFarland 0.5 density bacterial suspension obtained under sterile conditions was diluted to a density of 10^5 bacteria/25 µL with sterile normal saline.

Intravaginal E. coli Infusion

Rats were anesthesized [ketamine hydrochloride (75 mg/kg) and xylazine (15 mg/kg) intramuscular] and then were positioned on supine position. 100 µL adjustable automatic pipette was used for vaginal application. *E. coli* (25 µL, 1×10^5 CFU/rat) was withdrawn by adjusting the pipette at 25 µL. The pipette tip was advanced into the vagina and 25 µL *of E. coli* was slowly injected in front of the cervix. Care was taken to ensure that the pipette tip did not go into the urinary tract while the pipette was moving inside the vagina (Figure 2).

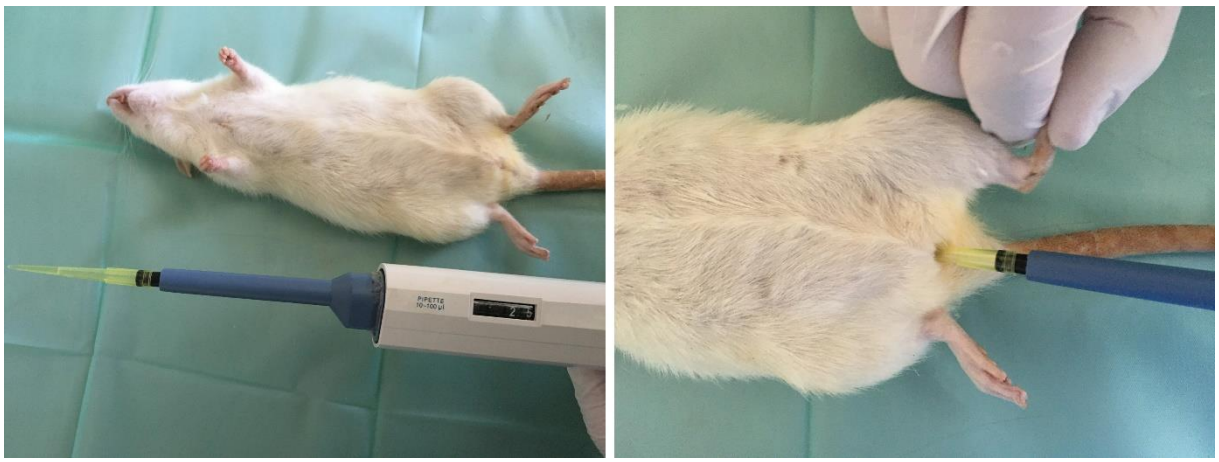

Figure 2. Intravaginal *E. coli* administration

Intrauterine E. coli Infusion

Rats were placed on supine position under ketamine hydrochloride (75 mg/kg) and xylazine (15 mg/kg) intramuscular] anesthesia, the operation area was shaved, and asepsis was achieved. The operation was performed by median incision. The abdominal cavity was reached by cutting the skin, muscle layers, and peritoneum. *E. coli* (25 µL, 1×10^5 CFU/rat) was precisely applied to the left cornu of the uterus by the apparatus attached to the micropipette tip as shown in Figure 3 and made sure that the solution was spread to other cornu. The cornu was returned to the uterine cavity after this procedure. The peritoneum and muscles were stitched with simple continuous sutures and the skin with horizontal U sutures, respectively.

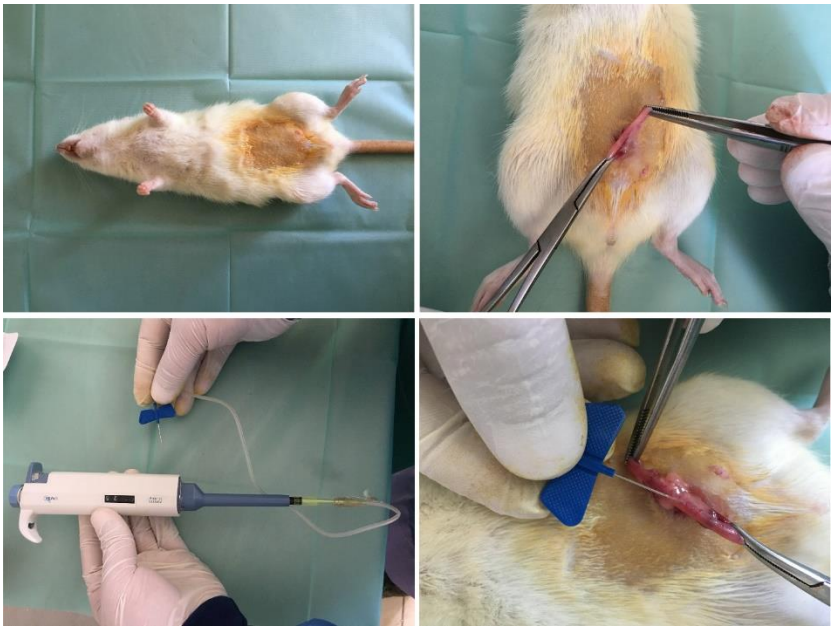

Figure 3: Intrauterine *E. coli* administration

Biochemical Analyses

Antioxidant-oxidant [malondialdehyde (MDA), glutathione (GSH)] and markers of inflammation (Endocan, Endoglin) were determined biochemically. GSH analyses were performed according to the method of Beutler et al. (1963) and MDA analyses were performed according to the method of Yoshoiko et al. (1979). Endocan and endoglin analyses were made using a commercial Enzyme-Linked ImmunoSorbent Assay (ELISA-Elabscience) kit.

Histopathological Analysis

The uterine tissues were kept in 10% buffered formaldehyde solution for 24 hours. The tissues were subjected to tissue processing in an automated device (Leica TP 1020). Sections of 5-µm thickness were taken from the tissues that were manually embedded in paraffin and the sections were stained with hematoxylin & eosin. The stained sections were evaluated under the light microscope (Olympus BX46) blindly for the presence of inflammation. Tissues containing polymorphonuclear leukocytes (PNL) were accepted as active inflammation in the evaluation of two serial cross-sectional areas at 400x magnification. The evaluation of the inflammation is scored by the pathologist subjectively using a two-step scoring system and both glandular and stromal components were considered. In the first step, the inflammation was evaluated by counting the numbers of the inflammatory cells as 0: no inflammation, 1: mild inflammation, 2: moderate inflammation, 3: severe inflammation. In the second step, the inflammation extensity was evaluated by a scoring system 0: no inflammation, 1: mild inflammation, 2: intermediate inflammation, 3: diffuse inflammation. The inflammation score (IS) was calculated by multiplying the scores taken from the first step and the second step (grade X extensity) resulting in a score range 0-9.

Statistical Analysis

The differences in biochemical parameters between the groups were determined through a one-way analysis of variance (ANOVA) method. The results were interpreted using Tukey's HSD test. The significance of the difference between histopathological scored data between the groups was evaluated with the Mann-Whitney U test. GraphPad package program (GraphPad Prism 8/San Diego, CA) was used for statistical analysis. The results were given as mean±standard deviation (SD) and $p<0.05$ was considered statistically significant.

RESULTS

Biochemical results

GSH was affected by *E. coli* infusion in the IVM or the IUM and the serum concentration decreased. There was a statistically significant decrease in GSH concentration in the IVM ($p=0.005$). and IUM ($p=0.03$). compared to the control (IVM-control $p=0.005$, IUM-control $p=0.03$). GSH concentration was not statistically different between IVM and IUM ($p>0.05$) (Figure 4A).

MDA was affected by intravaginal or intrauterine *E. coli* administration. Plasma MDA concentration increased significantly in both the IVM ($p=0.002$) and IUM ($p=0.001$) compared to the control group. MDA concentration was not statistically different between the IVM and IUM ($p>0.05$) (Figure 4B).

Endocan is shown in Figure 4-C and endoglin is shown in Figure 4-D. Both endocan ($p=0.02$) and endoglin ($p=0.03$) were statistically significant in the IUM compared to the control. There was a statistically significant increase in endoglin ($p=0.03$) whereas there was a numerical increase in endocan between the IVM and the control ($p>0.05$).

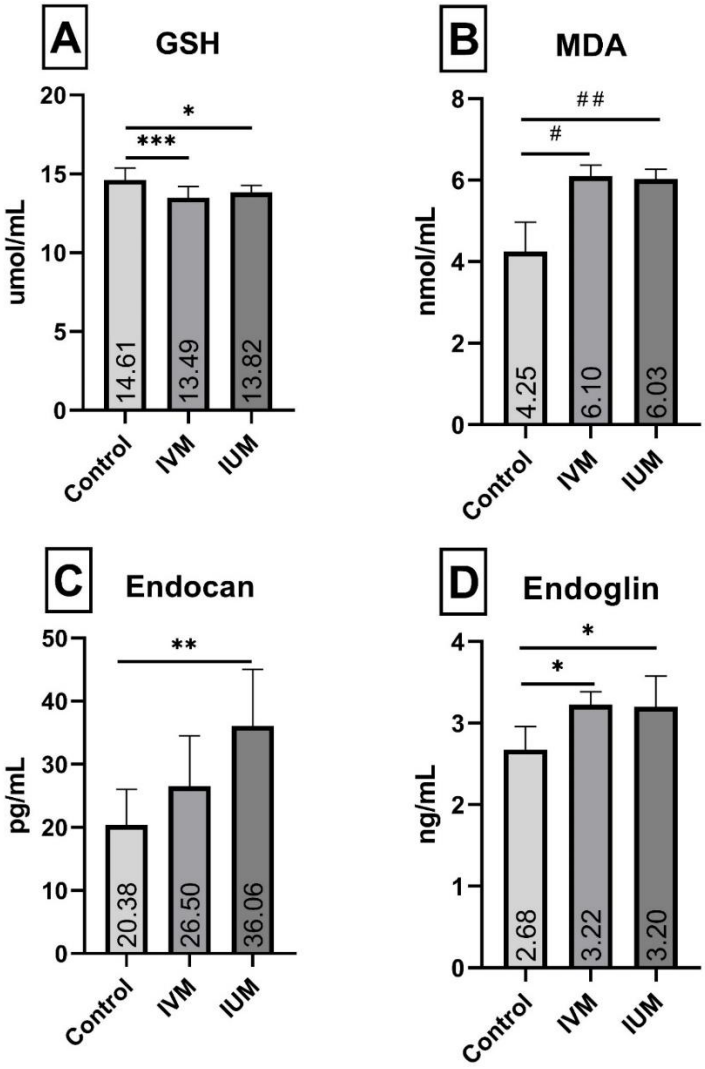

Figure 4. Means + SD of the three groups (A, B, C, D) for biochemical parameters. *p=0,03, **p=0,02, ***p=0,005, $^{\#}$p=0,002, $^{\#\#}$p=0,001

Histopathological results

Table 1 shows the median values of inflammation grade, inflammation extensity, and inflammation scores for the control, IVM, and IUM groups. Figure 5 presents the histological pictures of the groups. Inflammation grade, inflammation extensity, and inflammation score were found to be statistically significantly higher in the IUM compared to the control (p values 0.024, 0.009, and 0.001, respectively). The inflammation grade, inflammation extensity, and the inflammation score were found to be statistically significantly higher in the IVM compared to the control (p values 0.024, 0.024, 0.001, respectively). Finally, no statistically significant difference was found between the IUM and the IVM in terms of inflammation grade, inflammation extensity, and inflammation score (p values 1,000, 1,000, 0.728, respectively).

Table 1. Median values of inflammation grade, inflammation extensity, and inflammation score of control, IVM, and IUM.

Groups	Inflammation grade	Inflammation extensity	Inflammation score
Control	1	1	1
IVM	2	2	3
IUM	2	2	3

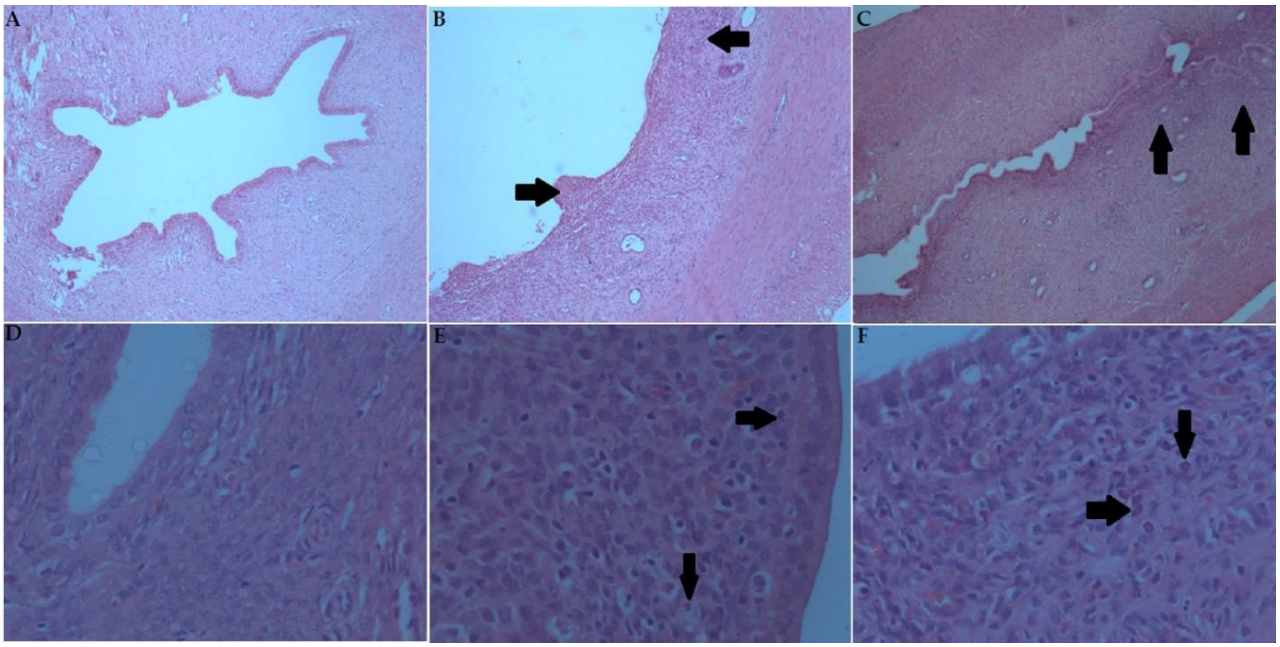

Figure 5. A: Endometrium of control tissue (H&E, 40x), B: Endometrium of intrauterine administration with inflammation, black arrows show inflammation (H&E, 40x), C: Endometrium of intravaginal administration with inflammation, black arrows show inflammation (H&E, 40x), D: Endometrium of control tissue (H&E, 400x), E: Endometrium of intrauterine administration with inflammation, black arrows show inflammatory cells (H&E, 400x), F: Endometrium of intravaginal administration with inflammation, black arrows show inflammatory cells (H&E, 400x).

DISCUSSION

Microorganisms usely detected in the endometrium are common bacteria (Cicinelli et al., 2008, 2009; Kitaya et al., 2017). The host organism can physiologically fight against these infections. Also a wide variety of immunocompetent cells, including macrophages, natural killer cells, and T lymphocyte subsets, infiltrate the endometrium throughout the menstrual cycle under physiological conditions. Endometritis is combated during tissue regeneration. Acute onset of endometritis continues as chronic endometritis unless it is physiologically prevented (Kitaya et al., 2018).

There are many experimental studies with regard to endometritis. Experimental studies with IUM are extensive (He et al., 2008; Nishikawa & Baba, 1985; Tiwari et al., 2018; Xiao et al., 2018). However, the experimental endometritis model with IVM, which is practical and easier in terms of application, is limited (Demirel et al., 2019). Both IVM and IUM were created and compared in our study. IVM is a new protocol and is easier to create than IUM. The IVM reliability was demonstrated by histopathologically evaluating neutrophil infiltration with oxidative stress and inflammation markers as reported previously.

Physiological ROS plays an important role in regulating many reproductive processes such as folliculogenesis, oocyte maturation, implantation, embryogenesis, and fetal-placental development. ROS are proinflammatory mediators that modulate cell proliferation in chronic cases of endometritis (Ngô et al., 2009). ROS may directly or indirectly damage macromolecules by activating signaling pathways (Roberts et al., 2009). ROS increase causes GSH and NADPH depletion and thus, a decrease in plasma level (Franco et al., 2007). GSH level in the IVM and IUM showed a significant decrease compared to the control in our study. The decrease in GSH shows an increase in ROS activity. ROS increase is also known to increase in inflammation and infectious conditions (Franco et al., 2007). This condition supports IVM by showing similar oxidative/antioxidative results

compared to IUM. In addition, an increase in GSH is known in the case of toxicity and inflammation of the uterus and ovary (Kaygusuzoglu et al., 2018). One of the ROS species is hydrogen peroxide (H_2O_2). H_2O_2 is a more stable compound than ROS. Therefore, it plays an important role in cell signaling, especially in response to stress. Hydroxyl radicals produced by H_2O_2 at high levels attack polyunsaturated fatty acids in the membranes. Thus, it causes lipid peroxide formation, which leads to widespread damage and eventually membrane fragmentation. Lipid peroxides decompose to form MDA, often used as an analytical marker of lipid peroxidation. An increase in MDA level is formed due to lipid peroxidation (Mazaheri-Tirani & Madadkar Haghjou, 2019). Our study found a statistically significant increase in both IVM and IUM compared to the control. This increase may be due to lipid peroxidation. The increase in both methods suggests that there is no difference between the two methods and supports IVM as a reliable model. Another study reported an increase in MDA levels in uterine and ovarian injuries (Kaygusuzoglu et al., 2018) as was also the case in our study.

Endocan-specific (endothelial cell-specific) molecule 1 (ESM-1) is a proteoglycan secreted by vascular endothelial cells (Sarrazin et al., 2006). It plays an important role in regulating the endothelial cell due to inflammation and in the functioning of lymphocytes (Afsar et al., 2014). It can be used as a biomarker for inflammation in many diseases (Balta et al., 2015; Sarrazin et al., 2006). The physiopathology of endometritis is characterized by inflammation of endometrial glandular and stromal tissues (Cohen et al., 2002). Our study has shown an increase in inflammation-related endometritis levels in our models. Therefore, it is predicted that there may be a relationship between endometritis and endocan concentration and it may be a biomarker of genital tract infections.

Endoglin is a proliferation-related, hypoxia-induced protein, mostly expressed in angiogenic endothelial cells (Baydın & Akbulut, 2013). Endoglin is expressed at low or even absent in normal endothelial cells whereas it is expressed at high rates in vascular endothelial cells in active local angiogenesis, inflamed tissues, and tumors inside and tumors around during embryogenesis (Ten Dijke et al., 2008). Our study found a statistically high level of inflammation in both IVM and IUM compared to the control. In particular, the fact that IVM was not different from IUM as shown by the results of inflammation due to *E. coli*.

Endometritis is a disease that causes inflammation in uterine tissue due to bacterial infections (Ness & Brooks-nelson, 2000). Demirel et al. (2019) histopathologically showed inflammation in the uterus in the experimentally developed endometritis model. Our study has also determined inflammation in both IVM and IUM. These results proved IVM as usable model.

CONCLUSION

IVM was determined by both biochemical analyses and histopathological analyses. It has been demonstrated biochemically and histopathologically that there is no difference between IVM and IUM. Complications that may occur during the application of IUM may adversely affect the studies to be conducted. The application of the operative method is a difficult method that requires both a surgeon and is time-consuming. In conclusion, both easy procedures in induction and uncomplicated intravaginal administration make IVM more suitable method for an experimental protocol.

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Ethical approval:

The experimental procedures were approved by the Kafkas University Animal Experiments Local Ethics Committee before starting the study (KAU-HADYEK 2020-071).

Conflict of interest: The authors declare that they have no competing interests

REFERENCES

Adnane, M., Kaidi, R., Hanzen, C., & England, G. C. W. (2017). Risk factors of clinical and subclinical endometritis in cattle: A review. *Turkish Journal of Veterinary & Animal Sciences*, *41*, 1–11.

Afsar, B., Takir, M., Kostek, O., Covic, A., & Kanbay, M. (2014). Endocan: A new molecule playing a role in the development of hypertension and chronic kidney disease? *The Journal of Clinical Hypertension*, *16*(12), 914–916. https://doi.org/10.1111/jch.12440

Balta, S., Mikhailidis, D. P., Demirkol, S., Ozturk, C., Celik, T., & Iyisoy, A. (2015). Endocan: A novel inflammatory indicator in cardiovascular disease? *Atherosclerosis*, *243*(1), 339–343. https://doi.org/10.1016/j.atherosclerosis.2015.09.030

Baydın, P. Ö., & Akbulut, H. (2013). Designing gene therapy vectors targeting tumor cell endothelium. *TURKISH JOURNAL OF ONCOLOGY*, *28*(4), 179–186.

Beutler, E., Duron, O., & Kelly, B. (1963). Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.*, *61*, 882–888.

Cicinelli, E., De Ziegler, D., Nicoletti, R., Colafiglio, G., Saliani, N., Resta, L., Rizzi, D., & De Vito, D. (2008). Chronic endometritis: Correlation among hysteroscopic, histologic, and bacteriologic findings in a prospective trial with 2190 consecutive office hysteroscopies. *Fertility and Sterility*, *89*(3), 677–684. https://doi.org/10.1016/j.fertnstert.2007.03.074

Cicinelli, E., Ziegler, D. D., Nicoletti, R., Tinelli, R., Saliani, N., Resta, L., Bellavia, M., & Vito, D. D. (2009). Poor reliability of vaginal and endocervical cultures for evaluating microbiology of endometrial cavity in women with chronic endometritis. *Gynecologic and Obstetric Investigation*, *68*(2), 108–115. https://doi.org/10.1159/000223819

Cohen, C. R., Manhart, L. E., Bukusi, E. A., Astete, S., Brunham, R. C., Holmes, K. K., Sinei, S. K., Bwayo, J. J., & Totten, P. A. (2002). Association between mycoplasma genitalium and acute endometritis. *The Lancet*, *359*(9308), 765–766. https://doi.org/10.1016/S0140-6736(02)07848-0

Cora, M. C., Kooistra, L., & Travlos, G. (2015). Vaginal cytology of the laboratory rat and mouse: Review and criteria for the staging of the estrous cycle using stained vaginal smears. *Toxicologic Pathology*, *43*(6), 776–793. https://doi.org/10.1177/0192623315570339

Demirel, M. A., Han, S., Tokmak, A., Gokay, N. E., Uludag, M. O., Ustun, T. Y., & Cicek, A. F. (2019). Therapeutic effects of resveratrol in Escherichia coli-induced rat endometritis model. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *392*(12), 1577–1589.

Franco, R., Schoneveld, O., Pappa, A., & Panayiotidis, M. (2007). The central role of glutathione in the pathophysiology of human disease. *Archives of Physiology and Biochemistry*, *113*, 234–258. https://doi.org/10.1080/13813450701661198

Garcia, L. (2010). Preparation of routine media and reagents used in antimicrobial susceptibility testing. In *Clinical Microbiology Procedures Handbook* (3rd Edition, p. p 181-201). ASM Press.

Güralp, O., Acikgöz, S., Tüten, N., Ekmekci, H., Schild-Suhren, M., Malik, E., & Tüten, A. (2020). Evaluation of serum endocan levels in endometriosis: A case-control study: Evaluation of serum endocan levels in endometriosis: A case-control study. *La Clinica Terapeutica*, *171*(6).

Hawinkels, L. J. A. C., Kuiper, P., Wiercinska, E., Verspaget, H. W., Liu, Z., Pardali, E., Sier, C. F. M., & Dijke, P. ten. (2010). Matrix metalloproteinase-14 (MT1-MMP)–mediated endoglin shedding inhibits tumor angiogenesis. *Cancer Research*, *70*(10), 4141–4150. https://doi.org/10.1158/0008-5472.CAN-09-4466

He, C., Wu, R., Li, L., Wang, X., Wang, J., Wang, S., Liu, W., & Yang, K. (2008). Establishment of a rat model of endometritis and its treatment with a Chinese-Western medicine emulsion. *Acta Laboratorium Animalis Scientia Sinica*, *3*.

Herath, S., Lilly, S. T., Fischer, D. P., Williams, E. J., Dobson, H., Bryant, C. E., & Sheldon, I. M. (2009). Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F2α to prostaglandin E2 in bovine endometrium. *Endocrinology*, *150*(4), 1912–1920.

Kaygusuzoglu, E., Caglayan, C., Kandemir, F. M., Yıldırım, S., Kucukler, S., Kılınc, M. A., & Saglam, Y. S. (2018). Zingerone ameliorates cisplatin-induced ovarian and uterine toxicity via suppression of sex hormone imbalances, oxidative stress, inflammation and apoptosis in female wistar rats. *Biomedicine & Pharmacotherapy*, *102*, 517–530. https://doi.org/10.1016/j.biopha.2018.03.119

Kitaya, K., Matsubayashi, H., Takaya, Y., Nishiyama, R., Yamaguchi, K., Takeuchi, T., & Ishikawa, T. (2017). Live birth rate following oral antibiotic treatment for chronic endometritis in infertile women with repeated implantation failure. *American Journal of Reproductive Immunology*, *78*(5), e12719. https://doi.org/10.1111/aji.12719

Kitaya, K., Takeuchi, T., Mizuta, S., Matsubayashi, H., & Ishikawa, T. (2018). Endometritis: New time, new concepts. *Fertility and Sterility*, *110*(3), 344–350. https://doi.org/10.1016/j.fertnstert.2018.04.012

Kuru, M., Öğün, M., Kulaksız, R., Kükürt, A., & Oral, H. (2018). Comparison of oxidative/nitrosative stress, leptin and progesterone concentrations in pregnant and non-pregnant abaza goats synchronized with controlled internal drug release application.

Kafkas Üniversitesi Veteriner Fakültesi Dergisi, *24*(6), 887–892.

Laloglu, E., Kumtepe, Y., Aksoy, H., & Yilmaz, E. P. T. (2017). Serum endocan levels in endometrial and ovarian cancers. *Journal of Clinical Laboratory Analysis*, *31*(5), e22079. https://doi.org/10.1002/jcla.22079

Mazaheri-Tirani, M., & Madadkar Haghjou, M. (2019). Reactive oxygen species (ROS), Total antioxidant capacity (AOC) and Malondialdehyde (MDA) make a triangle in evaluation of Zinc stress extension. *Journal of Animal and Plant Sciences*, *29*.

Mitsui, H., Shibata, K., Mano, Y., Suzuki, S., Umezu, T., Mizuno, M., Yamamoto, E., Kajiyama, H., Kotani, T., Senga, T., & Kikkawa, F. (2013). The expression and characterization of endoglin in uterine leiomyosarcoma. *Clinical & Experimental Metastasis*, *30*(6), 731–740. https://doi.org/10.1007/s10585-013-9574-9

Mohammed, Z. A., Mann, G. E., & Robinson, R. S. (2019). Impact of endometritis on post-partum ovarian cyclicity in dairy cows. *The Veterinary Journal*, *248*, 8–13. https://doi.org/10.1016/j.tvjl.2019.03.008

Ness, R., & Brooks-nelson, D. (2000). 29—Pelvic Inflammatory Disease. In M. B. Goldman & M. C. Hatch (Eds.), *Women and Health* (pp. 369–380). Academic Press. https://doi.org/10.1016/B978-012288145-9/50033-4

Ngô, C., Chéreau, C., Nicco, C., Weill, B., Chapron, C., & Batteux, F. (2009). Reactive oxygen species controls endometriosis progression. *The American Journal of Pathology*, *175*(1), 225–234. https://doi.org/10.2353/ajpath.2009.080804

Nishikawa, Y., & Baba, T. (1985). Effects of ovarian hormones on manifestation of purulent endometritis in rat uteruses infected with Escherichia coli. *Infection and Immunity*, *47*(1), 311–317.

R**oberts, R. A., Laskin, D. L., Smith, C. V., Robertson, F. M., Allen, E. M. G., Doorn, J. A., & Slikker, W. (2009).** Nitrative and oxidative stress in toxicology and disease. *Toxicological Sciences*, *112*(1), 4–16. https://doi.org/10.1093/toxsci/kfp179

Salah, N., & Yimer, N. (2017). Cytological endometritis and its agreement with ultrasound examination in postpartum beef cows. *Veterinary World*, *10*(6), 605–609. https://doi.org/10.14202/vetworld.2017.605-609

Sarrazin, S., Adam, E., Lyon, M., Depontieu, F., Motte, V., Landolfi, C., Lortat-Jacob, H., Bechard, D., Lassalle, P., & Delehedde, M. (2006). Endocan or endothelial cell specific molecule-1 (ESM-1): A potential novel endothelial cell marker and a new target for cancer therapy. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, *1765*(1), 25–37. https://doi.org/10.1016/j.bbcan.2005.08.004

Sheldon, I. M., Cronin, J., Goetze, L., Donofrio, G., & Schuberth, H.-J. (2009). Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. *Biology of Reproduction*, *81*(6), 1025–1032.

Sheldon, I. M., & Dobson, H. (2004). Postpartum uterine health in cattle. *Animal Reproduction Science*, *82–83*, 295–306. https://doi.org/10.1016/j.anireprosci.2004.04.006

Sheldon, I., Noakes, D., Rycroft, A., Pfeiffer, D., & Dobson, H. (2002). Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. *Reproduction (Cambridge, England)*, *123*, 837–845. https://doi.org/10.1530/rep.0.1230837

Ten Dijke, P., Goumans, M.-J., & Pardali, E. (2008). Endoglin in angiogenesis and vascular diseases. *Angiogenesis*, *11*(1), 79–89.

Tiwari, A., Singh, P., Jaitley, P., Sharma, S., Prakash, A., Mandil, R., Choudhury, S., Gangwar, N. K., & Garg, S. K. (2018). Eucalyptus robusta leaves methanolic extract suppresses inflammatory mediators by specifically targeting TLR4/TLR9, MPO, COX2, iNOS and inflammatory cytokines in experimentally-induced endometritis in rats. *Journal of Ethnopharmacology*, *213*, 149–158. https://doi.org/10.1016/j.jep.2017.10.035

Wiesenfeld, H., & Paavonen, J. (2010). 6—Pelvic Inflammatory Disease. In S. A. Morse, R. C. Ballard, K. K. Holmes, & A. A. Moreland (Eds.), *Atlas of Sexually Transmitted Diseases and AIDS (Fourth Edition)* (pp. 94–110). W.B. Saunders. https://doi.org/10.1016/B978-0-7020-4060-3.00006-5

Xiao, H.-B., Sui, G.-G., Lu, X.-Y., & Sun, Z.-L. (2018). Elevated levels of ADMA are associated with lower DDAH2 and higher PRMT1 in LPS-Induced endometritis rats. *Inflammation*, *41*(1), 299–306. https://doi.org/10.1007/s10753-017-0687-1

Yoshioka, T., Kawada, K., Shimada, T., & Mori, M. (1979). Lipid peroxidation in maternal and cord blood and protective mechanisms. *Am J Obstet Gynecol*, *135*, 972–976.