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Study the expression level of Annexin A2 gene in Oviduct Epithelial Cells of Iraqi Cows with Endometritis

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Abstract

The present study carried out to study the Annexin A2 gene Expression in oviduct epithelial cells of Iraqi cows with endometritis in comparison with it in the oviduct epithelial cells of healthy cows. Fifty uterine sample were collected from cows with macroscopic change in the uterus, samples were divided into two groups (molecular and histopathology) group . the samples were taken from oviduct ampulla immediately plunged into liquid nitrogen; and stored at−80 ◦C until the molecular study. The histological examination of 50 endometrial samples of the cows reproductive tract revealed that 30 samples exhibited histological changes associated with endometritis, while the 20 samples were normal endometrium . The molecular study involved RNA extraction from oviduct tissue ampulla and RNA concentration measurement by Quantus™ Fluorometer , cDNA syntheses and Quantitative Reverse transcriptase PCR (RT-qPCR) Preparation, The gene expression analysis of ANNEXIN A2 using RT-qPCR technology revealed a significant up-regulation in cows oviduct epithelial cell ampulla within endometritis compared to oviduct epithelial cells ampulla of normal endometrium cows (p-value< 0.0022) and significantly different ($P < 0.01$) the expression level (fold change) in oviduct with endometritis cows was (1.969) whereas oviduct with normal endometrium cows (1.076). In conclusion ,the result indicated that presence of gross lesion changes does not be necessarily indicate microscopic pathological change, Annexin A2 up-regulation in oviduct epithelial cells ampulla of cows with endometritis. This study identified potential marker gene for fertility in dairy cattle.

Keywords : Cow, Endometritis, Oviduct, Epithelial Cell, Annexin A2,gene.

Introduction

One of the main reasons for infertility and financial losses in the dairy industry is inflammation of uterine endometrium called endometritis, which frequently occurs after calving are diagnosed between 3 and 5 weeks postpartum [1]. There are several type of pathogenic bacteria lead to development cow endometritis including Staphylococcus, Streptococcus, Escherichia, Corynebacterium, Pseudomonas, Proteus, Necrobacillus, Pseudomonas aeruginosa, Campylobacter genitalia, Haemophilus, Bacillus pyogenes, Bacterium burgeri, Trueperella, Fusobacterium, and Prevotella, [2].the classification of cow endometritis to clinical endometritis characterized with purulent vaginal discharge without systemic illness and subclinical endometritis without any clinical sign [3] .oviduct is small tubular structure linked uterus with ovary and it is important in many reproductive events such as fertilization, oocyte final maturation and early embryo development[4] anatomically contain three regions, isthmus, ampulla and infundibulum [5] oviduct epithelial cells play important role for fertility because it is prolong the life span of sperm, and the sperm binds with the ova in oviduct[6], so any problem in oviduct causes infertility [7] ,the one of main protein in cow oviduct is Annexin A2 gene the function of Annexin A2 gene in cow

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oviduct linked with binding of sperm in epithelial cell of oviduct and formation functional reservoir[8] its found the Annexin A2 gene have function in interaction between gamete , early embryo with oviduct epithelial cell is crucial for fertilization and early embryo development [9] Annexin A2 protein coding gene belong to Annexin family is a multifunctional calcium2+ (Ca2+) and phospholipid-binding protein[10] ,play important role in regulation inflammatory response and the infection process of bacteria, viruses, and other pathogens [11],Annexin A2 play integrant role in adhesion of bacteria and in absent of Annexin A2 there is decrease of bacterial adhesion[12] , During infection facilitates the adhesion and internalization of bacteria and viruses elevate their replication and release, which is harmful effect on the host [13]. The present study carried out to study the Annexin A2 gene Expression in Oviduct epithelial cells of Iraqi cows with endometritis in comparison with it in the oviduct epithelial cells of healthy Iraqi cows.

Material and Methods

Ethical approved

Ethical approval was granted according to the committee for Ethical Scientific Research at the College of Veterinary Medicine, University of Al-Qadisiyah (Approval number P.G/872 at 24/2/2025).

Sample collection

Collected all the research samples, total 50 uterine samples with uterine macroscopically change, from the female reproductive system of cows slaughtered at the slaughterhouse in al-qadisiyah governorate. The collection was conducted twice a week for five months, from 1/10/2024 to 1/2/2025. The samples were taken from endometrium and oviduct ampulla .The sample was divided into two groups (molecular and histopathology) groups . The histological and molecular study were done in the labs of the Veterinary Medicine College / University of Al-Qadisiyah .

Samples for molecular study

Samples were taken from cows' oviducts, and sealed in a plastic bag, and transported by box containing ice. They were cleaned from blood clots and extra tissues by washing in phosphate buffer saline (PBS). Then, a Surgical scalpel was used to extract 3cm3 from the ampulla, and the sample was put in a Cryovial tube to store in a liquid nitrogen tank until further processing (Gene Expression).isolation of oviduct epithelial cell protocols According to[14].

Samples for histopathology study

The samples were taken from the cow's endometrium, used a surgical scalpel to cut 1cm3 from the uterine endometrium layer washed in normal saline, and put in a cup filled with buffer formalin 10%. According to[15], the sample preparation for histological sectioning according to [16] and [17] methods,50 sample(1cm^3) were taken from cow uterine endometrium , sections of endometrial specimens for light microscopy, 5-7 µm thick, were stained with Hematoxylin and Eosin .

RNA extraction and cDNA synthesized

For Annexin A2 and GAPDH genes, a set of primer was synthesized by Macrogen (South Korea) according to.[18] table (1).total RNA extraction according to the manufacturer's protocol using a silica gel column-based spin column method using the kit ADDBIO/Korea kit , RNA concentration measurement by Quantus™ Fluorometer (Promega, USA). cDNA synthase using the kit from ADDBio (Korea) , The relative expres¬sion of genes of interest was normalized using GAPDH gene as a ref¬erence gene and calculated using the 2-ΔΔCt method Schmittgen and Livak.[19], Quantitative real-time polymerase chain reaction (qPCR) was performed using Real time qPCR machine (biorad /USA) using AddScript RT-qPCR Syber master (AddBio, Korea).

Statistical analysis

Statistical analyses were performed using the Graph Pad Prism software v10.4.1.gene expression level between oviduct ampulla of cow with endometritis and healthy endometrium groups were analysis using unpaired t-test, mean ,STD. Differences with $p < 0.01$ were considered sta¬tistically significant. According to.[14].

Result

The histological examination of 50 endometrial samples from the cows reproductive tract revealed that 30 samples exhibited histological changes associated with endometritis, while the 20 samples were normal endometrium ,the histological study of the endometrium in cows revealed several tissue change confirming the presence of endometritis the observed histological changes the glandular part of endometrium clear sloughing of epithelium. The epithelial cells show necrotizing process liquefactive necrosis and severe damage in the endometrium myofiber layer. with infiltration of inflammatory cells (mainly macrophages).Figure (1)

Gene expression ANNEXIN A2

The gene expression analysis of ANNEXIN A2 using RT-qPCR technology revealed a significant up-regulation in cows oviduct epithelial cells ampulla within endometritis compared to oviduct epithelial cells ampulla of normal endometrium cows (p-value< 0.0022) and significantly different ($P < 0.01$) the expression level (fold change) in oviduct with

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endometritis cows was (1.969) whereas oviduct normal endometrium cows (1.076) with a mean difference (0.8935 ± 0.2764 mean ± SEM).Figure (2)

Table 1. Real-time PCR primers

Gene	Primer	sequence 5'-3'	References
GAPDH	Forward	ACCCAGAAGACTGTGGATGG	[18]
	Reverse	ACGCCTGCTTCACCACCTTC	
Annexin A2	Forward	CGTGCTCCAGCTAACAGTTCT	[18]
	Reverse	GGAAAGCCAGGTAATGCGTA	

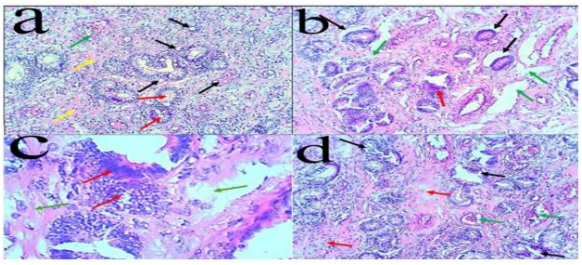

Figure 1. histological section of cow endometrium .a The glandular part of endometrium shows vacuolations in the epithelial cells (Black arrows) with narrowing of glands lumen (Red arrows). The section shows clear blood vessels congestion (Green arrows). The section shows severe damage in the endometrium myofiber layer (Yellow arrows).b The glandular part of endometrium shows clear sloughing of epithelium from glandular basement membrane (Black arrows). The epithelial cells show necrotizing process (liquefactive necrosis, Red arrow). The section shows severe damage in the endometrium myofiber layer (Green arrows). C The epithelial cells show necrotizing process (liquefactive necrosis, Red arrows). The section shows severe damage in the endometrium myofiber layer (Green arrows).d The glandular part of endometrium shows necrotizing lesion (Black arrows) in the epithelial cells and myofibers layer (Red arrows) with infiltration of inflammatory cells (mainly macrophages). The section shows clear blood vessels congestion (Green arrows).

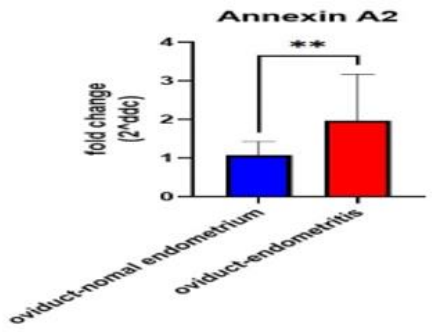

Figure 2. showed up-regulation of Annexin A2 gene expression in oviduct epithelial cell ampulla with endometritis compared with oviduct epithelial cell ampulla of normal endometrium cows (p-value <0.0022)

Discussion

The histological examination of 50 endometrial samples from the cows reproductive tract revealed that 30 samples exhibited histological changes associated with endometritis, while the 20 samples were normal endometrium. The histological study showed the presence of severe damage in the endometrium myofiber layer ,the glandular part of endometrium shows clear sloughing of epithelium necrotizing process liquefactive necrosis , The glandular part of endometrium shows vacuolations in the epithelial cells blood vessels congestion infiltration of inflammatory cells mainly macrophages this result agreed with [20] which show that necrobiosis of the epithelial layer of the mucosa, cellular infiltration with shaped elements of blood in the functional layer, swelling of the cells of the uterine gland edema of the stroma of the functional layer of the endometrium, swelling of the epithelial layer of the endometrial mucosa, moreover, the presence of gross lesions does not necessarily indicate the presence of Histopathological changes ,this observation agreed with the study of [21]in female camels which mention that the gross examination of 247 samples from female reproductive tract revealed that only 67 exhibited macroscopic pathological change, and among them only 25 samples tested positive.

The results of the gross and histological lesion examination of samples reveal that there is several factors may effect on the gross and histological lesion examination like truma, light during slaughter, animal injuries, or increased blood flow , congestion due to vascular pressure and physiological state of female reproductive system .

The present study using RT-qPCR technology showed up-regulation in gene expression in oviduct epithelial cell ampulla of cows with endometritis fold change 1.969 compared with oviduct epithelial cell ampulla cows with normal endometrium fold change 1.076 with a mean difference (0.8935 ± 0.2764 mean ± SEM) p value <0.0022, this result agree with the results of [22]which study Annexin A2 in cow endometrium which show that there is strong positive correlation with bacterial infection of endometrium and found there is up-regulation in cow with endometritis in his studies by using 2D gel electrophoresis and mass spectrometry while in present study used RT-PCR because it is more

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sensitive and provide quantitative measurement of RNA level, Annexin A2 is present in the cytoplasm and on cell surfaces this gene can bind membranes to other membranes for reason called bridge (Annexin A2) [23],during inflammation ANNEXIN A2 modulates the nuclear factor kappa-B (NF-κB) and cell apoptosis signaling pathways and have role in guide the chemotaxis of inflammatory cells toward inflammation sites Which contributes to increased inflammation [24] inflammatory cell secretion in oviduct It has a negative effect on oviduct environment and effect on fertilization process ,Annexin A2 role chemotaxis attract of pathogen to site infection facilitate their invasion and proliferation , the female genital contain bacteria also in oviduct [25,26,27] , role in proliferation of this bacteria and increase infection ,inflammation reduce oocyte quality and causes damage in zona pellucida [28,29] impair oocyte wall and chemotaxis effect of ANNEXIN A2 can contribute attract of sperm toward oocyte lead to polyspermy and failure of fertilization , [30]and [31] found that ANNEXIN A2 in human and mice can contribute causes fibrosis in lung and liver according to that it may be have the same role in the oviduct fibrosis formation lead to obstruction and impair oocyte and sperm movement through oviduct and possible permanent infertility when Annexin a2 increase, the increase gene expression of ANNEXIN A2 in the oviduct is affected by endometritis and that inflammation has extended to oviduct ampulla, the expression of ANNEXIN A2 acts as a double edged sword it crucial role for sperm-oocyte adhesion and enhances fertilization rate , and the presence of inflammation it turns to negative effect by attracting inflammatory cell to site of infection and lead to chronic inflammation.

Conclusion

The presence of macroscopic lesion in the uterus does not necessarily as indicator for microscopic pathological change. Increased expression of the Annexin A2 in oviduct ampulla in cows with endometritis. Endometritis extends its effects on the oviduct by it is effect on the oviduct genes.

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Conflict of interest

No conflict of interest is found for the present study.

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