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The S100A10 Gene Expression in Oviduct Epithelial Cells of Iraqi Cows with Endometritis

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Abstract

THE PRESENT study carried out to study the S100A10 gene Expression in Oviduct epithelial cells of Iraqi cows with endometritis in comparison with it in the oviduct epithelial cells of healthy Iraqi cows. Fifty uterine sample were collected from cows with macroscopic change in the uterus, the samples were taken from uterine endometrium and oviduct ampulla, samples were divided into two Into parts, one for molecular examination and second part for histopathological study. The histological examination of 50 endometrial samples of the cow's 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 S100A10 using RT-qPCR technology revealed a significant up-regulation in cows oviduct ampulla within endometritis compared to normal endometrium cows (p-value< 0.0001) the expression level (fold change) oviduct epithelial cell in endometritis cows was (2.346) whereas in oviduct epithelial cell of normal endometrium cows (1.038) ,In conclusion ,the result indicated that presence of gross lesion changes does not be necessarily indicate microscopic pathological change ,up-regulation in S100A10 in oviduct ampulla of cows with endometritis. Endometritis extends its effects on the oviduct by it is effect on the oviduct genes.

Keywords: Cow, Endometritis, Oviduct, Epithelial cell, S100A10 gene.

Introduction

Cows breeding is important economic sources for, breed, meat, milks and their product and fertilizers [1], cow endometritis causes large economic lost by its effect on reproductive performs by lowering the rate of conception, delaying the service interval, and repeat breeding. The risk factors for endometritis in cattle include contamination during artificial insemination, dystocia, managing parturition in an hygienic manner, numerous births, and abortion [2], Even after the health problem appears to be under control, postpartum uterine infection and inflammation continue to negatively impact oocytes, embryo development, and the endometrium for at least three months[3], within five weeks of calving, up to 50% of dairy cows may get one or more forms of inflammatory disease of the reproductive system [4], clinical endometritis it is characterized by purulent vaginal discharge without any systemic illness, subclinical endometritis without clinical sign can only detected by endometrial sampling [5]. Oviduct is important reproductive structure, which joins the ovary and uterus take place oocyte final maturation, fertilization, and early embryonic development are all facilitated by the oviduct[6], anatomically contain three portions infundibulum, ampulla and isthmus, lined with epithelial cell[7], oviduct epithelial cells have important role in crucial in transport of gamete, sperm binding and early embryo development [8], can be ciliated or secretory cell ciliated cell distributed largely in ampulla and infundibulum [9] . S100A10 gene found in oviduct of cows it is believes to play a role in formation reservoir [10], S100A10 is protein coding form a heterotetrameric with Annexin A2 [11] S100A10 is dependent on Annexin A2 for intracellular survival; without annexin A2, it is quickly degraded by both independent and ubiquitin-dependent proteasomes. To enhance its affinity for Ca2+ and enable its involvement in Ca2+-dependent activities like

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membrane binding, Annexin A2 needs S100A10 [12], plays important role during inflammation by recruit the macrophage to site of infection [13]. The present study carried out to study the S100A10 gene Expression in Oviduct ampulla epithelial cells of Iraqi cows with endometritis in comparison with it in the oviduct epithelial cells of healthy Iraqi cows.

Material and Methods

Ethical approval

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

Total 50 uterine samples with uterine macroscopically change**,** from the female reproductive system of cows slaughtered at the slaughterhouse in Al-Qadisiyah governorate. The sample collection was conducted twice a week for five months**,** from 1/10/2024 to 1/2/2025**. The samples of the** female reproductive tract from the were taken uterine endometrium and oviduct ampulla. The samples were divided into two groups molecular group study and histopathology group study. The study at histological and molecular biology lab of the University of Al-Qadisiyah.

Sample for molecular study

Samples were taken from cows' oviducts, well-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 $3cm^3$ 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].

Sample for histopathology study

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

RNA extraction and cDNA synthesized

For S100A10 gene and GAPDH, 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 expression of genes of interest was normalized using GAPDH gene as a reference 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 conducted using GraphPad Prism software (v10.4.1). The gene expression levels between the oviduct ampulla of cows with endometritis and the healthy endometrium group were analyzed using an unpaired t-test. The results are presented as means and standard deviations (STD). Differences with a p-value of less than 0.01 were considered statistically significant [14].

Results

Histological study

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 changes 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). Fig.(1)

Gene expression S100A10

The gene expression analysis of S100A10 using RT-qPCR technology revealed a significant up-regulation in cows' oviduct epithelial cell ampulla within endometritis fold change (2.346) compared to oviduct epithelial cell of normal endometrium cows fold change (1.038) (p-value< 0.0001) and significantly different ($P < 0.01$) with a mean difference (1.308 ± 0.3156 mean ± SEM). All data analyses used un-paired t-test using graph pad prism program. Fig.(2)

Discussion:

The histological examination of 50 endometrial samples from the cow's reproductive tract revealed that 30 samples exhibited histological changes associated with endometritis, while the 20 samples were normal endometrium. The histological study showed ,revealed 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 lesion and histological examination of samples to the study may reveal that there is several factors may effect on the gross and histological 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 cells ampulla of cow with endometritis fold change 2.346 compared with oviduct epithelial cells cows with normal endometrium fold change 1.038 with a mean difference (1.308 ± 0.3156 mean ± SEM) p value <0.0001, [22] show that S100A10 gene that does not bind to calcium but bind with ANNEXIN A2 gene which plays an important role in the cellular localization, stability, and functionality up regulation of s100a10 association with ANNEXIN A2. During inflammation, extracellular matrix (ECM) degradation is a vital step and the S100A10 gene crucial molecule in the process S100A10 gene helps stimulate enzymes that break down ECM, allowing cells such macrophages to move through tissues [23] may be macrophage attack sperm as allogeneic and lead to chronic inflammation and the collagen main of ECM degradation lead to loss of cohesion oviduct epithelial cells ,and effect on cilia function impair oocyte and sperm movement during oviduct, facilitating communication between cells and their microenvironment to regulate cellular adhesion play role in adhesion sperm in oviduct epithelial cells.

Conclusion

The presence of macroscopic lesion in the uterus does not necessarily as indicator for microscopic pathological change. There is increased expression S100A10 in oviduct epithelial cells ampulla in cows with endometritis. Endometritis extends its effects on the oviduct by it is effect on the oviduct epithelial cells genes.

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Declaration of competing interest

The authors declare that there is no conflict of interest.

TABLE 1. Real-time PCR primers

Gene	Primer	sequence 5'-3'	References
GAPDH	Forward	ACCCAGAAGACTGTGGATGG	[18]
	Reverse	ACGCCTGCTTCACCACCTTC	
S100A10	Forward	GGATTTCTGAGCATATGGGACC	[18]
	Reverse	GAGCAAGAGGATGCAAGCAATA	

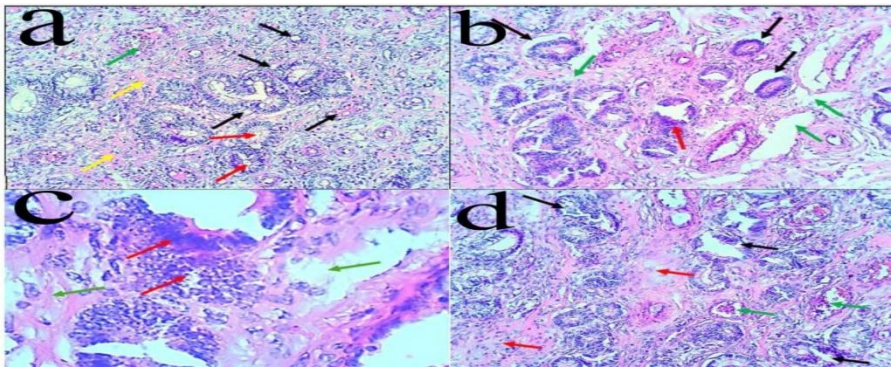

Fig.1. histological section of cow endometrium. A: the glandular part of endometrium shows vacuolations in the epithelial cells (Black arrows) with narrowing of glands lumen (Red arrows). The section shows clear blood vessels congestion (Green arrows). The section shows severe damage in the endometrium 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).

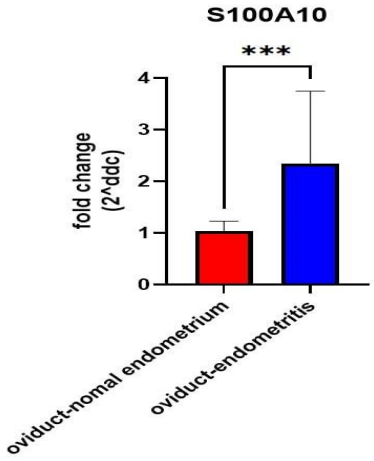

Fig.2. Showed up-regulation of S100A10 gene expression in oviduct epithelial cell ampulla with endometritis compared with oviduct epithelial cell ampulla of normal endometrium cows (p-value <0.0001).

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التعبير الجيني S100A10 في الخلايا الظهارية لقناة البيض لدى الأبقار العراقية المصابة بالتهاب بطانة الرحم

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الملخص

أُجريت هذه الدراسة لدراسة التعبير الجيني لجين S100A10 في الخلايا الظهارية لقناة البيض لدى الأبقار العراقية المصابة بالتهاب بطانة الرحم، ومقارنته بالخلايا الظهارية لقناة البيض لدى الأبقار العراقية السليمة. جُمعت خمسون عينة رحم من أبقار تعاني من تغيرات عيانية في الرحم، من بطانة الرحم وقناة البيض، وقُسِّمت العينات إلى مجموعتين: المجموعة الجزيئية ومجموعة الدراسة النسيجية. كشف الفحص النسيجي لخمسين عينة من بطانة الرحم في الجهاز التناسلي للأبقار أن ثلاثين عينة أظهرت تغيرات نسيجية مرتبطة بالتهاب بطانة الرحم، بينما كانت العينات العشرون سليمة. تضمنت الدراسة الجزيئية استخراج الحمض النووي الريبي من أمبولة أنسجة قناة البيض وقياس تركيز الحمض النووي الريبي بواسطة جهاز Quantus™ Fluorometer، وتوليف cDNA وإعداد تفاعل البوليميراز المتسلسل العكسي الكمي (RT-qPCR)، وأظهر تحليل التعبير الجيني لـ S100A10 باستخدام تقنية RT-qPCR ارتفاعًا كبيرًا في أمبولة قناة البيض للأبقار المصابة بالتهاب بطانة الرحم مقارنة بالأبقار ذات بطانة الرحم الطبيعية (القيمة الاحتمالية <0.0001) ومستوى تعبير مختلف بشكل كبير (P <0.01) (التغير في الطية) لخلايا قناة البيض الظهارية في الأبقار المصابة بالتهاب بطانة الرحم كان (2.346) بينما في خلايا قناة البيض الظهارية للأبقار ذات بطانة الرحم الطبيعية (1.038)، وفي الختام، أشارت النتيجة إلى أن وجود تغيرات آفة جسيمة لا يشير بالضرورة إلى تغير مرضي مجهري، والارتفاع في S100A10 في أمبولة قناة البيض للأبقار المصابة بالتهاب بطانة الرحم. يمتد تأثير التهاب بطانة الرحم إلى قناة فالوب من خلال تأثيره على جينات قناة البيض.

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