Studying the relationship between advanced oxidative stress of protein and uterine disturbance in dairy cows

Saeed Kazem Hassan[1*], Eftkar Mahdy Al-Najar[1]

[1] Al-Furat Al- Aawsat Technical University, Technical College of Al-Musaib, Department of animal production technology
***Corresponding author e-mail: Iftkaralnajar@atu.edu.iq**

Received:
Oct. 18, 2022

Accepted:
Nov. 22, 2022

Published:
Mar. 23, 2023

Abstract:

The study was conducted on 100 dairy cows in Tag-Alnahrain station, located in Al-Qadysia province, post parturition (after 40-60) days after calving. The aim of the study was to test the relationship between oxidative stress, particularly protein oxidation, and uterine health during the puerperium. The cows are divided into four groups according to intense clinical sighs of merit. In order to distinguish between degrees of inflammation, in this study, the cutoff value of PMN %. was set at 8% to distinguish between degrees of inflammation. The group (H) was (54). The SCE group consisted of 22 cows infected with subclinical endometritis, the EM1 group consisted of 11 cows infected with clinical endometritis Grade 1, and the EM2 group consisted of 13 cows infected with clinical endometriosis Grade 2. The advanced oxidation protein product (AOPP) was estimated in plasma and vaginal mucus discharges by using a commercial laboratory method to detect the dityrosin formation, which is the major content of AOPP. AOPP, a good marker of protein oxidation, was visualised by western blot,. The study demonstrated that there was an increase in plasma AOPP in the EM2 group with a significant value of p 0.01.The study also showed a significantly increased concentration of AOPP in vaginal mucus discharges in the group of animals infected with (SCE). The study clarified that there was an increase in neutrophil point age of more than 8% in vaginal mucus discharge after collecting mucus by metrecheek in the (EM1) group with a significant value of p≤0.0l while it was significantly(p≤0.01) in uterine mucus collected by cytobrush.

Keywords: protein oxidation, merits, sub clinical merits, oxidativestress.

Introduction

The main feature of cow production is reproduction, because many cows do not achieve peak reproductive performance, which results in significant loss [1].Studies show that 75% of postpartum disturbances in cattle occur during the first 30 days after parturition [2]. found that postpartum immune function, hormonal and metabolic functions, and energy balance were all compromised. Physiologically, the composi-

tion of uterine microflora changes over time and bacteria clear, but the risk of developing clinical merits remains elevated, particularly in cows that have twins, dystocia, retained placenta, or metabolic disorders [3]. more over the development of subclinical endometritis can impact negatively on fertility[4]. The presence of high neutrophil counts in the endometriums of cows affected by clinical or subclinical merits suggest the potential involved of oxidative stress especially protein oxidation among the mechanism of merits and that could compromise fertility [5]. The regulation of follicular fluid environment, follicular genesis, stenaidogeness, corpus lutein function, and autolysis in dairy cows is influenced by both oxidant and antioxidant balance[6]. In dairy cows, oxidative stress and protein oxidation are implicated in embryonic death [7]. Dairy cows produce the enzyme NADPH oxidase, which when activated produces superoxide o-2, a precursor to hydrogen peroxide (H2O2) and other reactive oxygen species (Ros). Hydrogen peroxide can react with myeloperoxidase (MPO) giving rise to strong oxidant short - Living intermediate capable of reacting with WI halides and giving highly microbiocidal species(hypochlorous acid) (HOCL) which is a potent bactericide molecule that can also modify extracellular targets and affect the function of neighbouring cells [8].(Hocl) attacks proteins, inducing alteration to protein backbone and sid chains. So it alters the protein function as receptors, enzymes, transporters, or structural proteins and generates new antigens [9]. The products of protein side – chain oxidation are preferable to assays because of their stable state and ease of detection. The most frequently used biomarker of protein oxidation is advanced oxidation protein product (AOPP), which may be considered the more stable marker of oxidative stress linked to phagocyte activity .Plasma profiles of the (AOPP) albumin ratio provide a sensitive indication of protein oxidation [10].[11] in dairy cows, pathogens are often introduced in uterus during the (AI) procedure and generate an inflammatory reaction to protein oxidation and (AOPP) generation. The development of subclinical endometritis is thought to be a common event in dairy cows after artificial insemination [12]. When uterine physical defences are breached, the next line of defence is represented by neutrophils, triggering an inflammatory process. The resulting activated leukocyte and vasoactive substances released during. Inflammation can increase blood vessel permeabity, resulting in plasma protein leaking into the endomaterial surface [13,14]. The goals of this study are to test (AOPP) as an inflammatory marker of protein oxidation in dairy cows affected by various stages of merit in order to better understand the role of protein oxidation in the pathophysiology of reproduction waste.

Materials and Methods

Animal of study

The study was carried out on (100) frozen tin dairy cows at TaG Al-Nahrain farm in Al-kadesia provine - lraq For the period March 2021 to March 2022. They received reproductive management by clinical check-up per formed soon after calving (20-40 day) though the reproductive information was collected from parturition and health records saved in the station . All of the cows suffer from uterine disorder after calving, so they were divided into four groups (H ,SCE , EM1 , EM2) according to mucous vaginal scour and uterine cytology, with the aid of a veterinary team working on the farm .

Clinica Tests

Data were gathered from records containing the following information: number, Aay, calving number, times of abortion, oestrus regulation, (Al) system, number of conceives, type and time of last calving, and reproductive problems and diseases infected with.

Samples Collection

Blood samples (5-10)ml were collected from the tail vein using evacuated EDTA tubes for plasma (AOPP) analysis by the manner of [15] by using kit another blood sample were collected in evacuated without EDTA tub for serum hormone concentration measure.

mucous secretion collected :

a- evaluation of vaginal mucous.

[16] performed the cytobrush evaluation by sorting the metricheek device into vagina. The cup of the device is sorted into the external uterine orifice . Then the opposite side . of the device is elevated slightly to fill the cup with vaginal mucus. the device was pulled gently from the vaginal mucus. was scored according to the studies of [16,17]. On a (0 to 3) scale (score 0 represents clear mucous ; score 1 represents mucus containing flecks of white or off-white PUS ; Score 2 represents mucus discharge containing 50% white or muco-purulent material ; and score 3 represents discharge containing discharge contain ≥50% purulent material, white or yellow but sometimes sanguineous.

b – Evaluation of endometrial cytology :

According to studies[18,19], endometrial cytology was performed using a cytobrush that was sterilised with formaldehyde gas before use.The external organs of reproduction and their margins were cleaned with benzalkumium and alcohol before the cytobrush was inserted into the vagina . When the cytobrush was passed through the cervix and inserted into the base of the uterine body under a rectal operation .The brush was exposed on the dorsal membrane of the uterine wall .The endometrial

samples were then collected by rotating (360 degrees Celsius) twice while in contact with the dorsal wall.

Cytology slides were prepared by rolling the cytobrnsh on three clean glass slides and immediately fixed with cytokeep (Nippon Shoji, Oska ,Japan). The slides were stained with Giemsa stain within 2 h after preparation. to assess endometrial inflammation .A minimum of 200 cells were counted at (400x) magnification for cytological assessment [16,20].

statistical analysis

study data was analyzed by using complete Randomized design (CRD) to know the effect of different treatment on the studied characteristics. significant differences were compared between their means by using [21] and by using SAS program [22].

Results and Discussion

The study showed that the percentage rate of merits in Tag- Alnahrian farm was 46% divided according to intense of infection into 22 cows classified under subclinical endometritis (SCE) 11 cow sunder Acute endometritis Grad 1(EM1) and (13) cows classified under Acute endometritis Grad 2 (EM2) this results improve the dangerous problem of infection with merits in cattle. The result in tab 1 is in agreement with that found by [23].

Table (1): Show the percentage of the distribution of groups according to the severity of inflammation in the sample of cows studied according to mucus secretion scale and PMN%

Groups	Number	Sample
H	54	54.00
SCE	22	22.00
EM1	11	11.00
EM2	13	13.00
Totel	100	%100
Chiseqner value	---	47.601**
P≤ (0.01)**		

The bacteriological test results revealed that each cow was infected with at least one genius or species of bacteria in uterine and vaginal fluid, as shown in Table (2).This is good that the bacterial contamination occurred in most animals of study and there was no relationship between bacterial merits and protein oxidation biomarker (AOPP) though the cow uterine was usually contaminated with multiple kinds of bacteria but didn't develop to clinical merits [24] while the cow bacterial infection, especially with E. coli and trueperel pyogenic result from bacterial isolation was of the morpathogenic type . This finding agreed with the findings of [11,25].

Table (2): cows positive for bacterial species and cultivars isolated by bacteriological culture from uterine and vaginal swab

Raterial crouns/species	H(N*) =54	SCE(N*) =22	EM1n* =11	EM2n = 13
Pervotel pyogen	0	-	2	4
S. hacmolitncus	1	0	3	-
S.cpidermidis Bacillus spps	1	0	2	-
E.coli	1	0	4	1
Micro coccus Leuteus	0	3	0	1
Antero coccus	1	2	1	1
Proteus	1	1	1	1
Polymicrobism	5	6	11	8

As shown in tab (3), the study found that plasma AOPP of group (EM2) was significantly higher (p 0.01).This result agree with that found by[11,26] The study found a significantly higher AOPP p 0.01 in vaginal mucus of SCE groups of cows that did not demonstrate any clinical sigh of merit and had a mucus score of more than 2 and PMN% greater than the threshold cut of PMN 8%.This means that the AOPP is the best biomarker for SCE that always causes repeat breeders and failure ovulation also effects on reproductive performance. The AOPP was increased as a result of neutrophil activation during uterine infection early postpartum or once through the first conception and represents useful markers of MPO/ HOCL protein oxidation [26].

Table (3): The concentrations of AOPP in blood plasma and vaginal mucus

AOPP Protein (ng/mg)	H	SCE	EM1	EM2	Morale leved
Plasma blood	5.7 C ±25	6.8 B ±76	0.9 B± 80	4.3 A±90	0.0006 **
Vaginal (VM) Mucus	0.4 ±8.2 C	0.6 ±32 A	1.6 ±22.1 B	0.4 ±11.9 D	0.0001 **
the averages that carry different letters with in the same row differ significantly between them **P ≤ (0.01) .					

The study showed a significantly increased ($p<0.01$) in neutrophil proportion (22.3± 0.95) in the (EM1) animal group, shown in Tab4. This finding is consistent with that of [25].

Table (4): the percentage of neutrophils in uterine fluids and vaginal mucus using metrcheck devig and brush

Neutrophil count %	H	SCE	EM1	EM2	Morale leved
Metrcheck Devig	4.5 ±0.16 C	18.7 ±1.03 Ab	22.3 ±0.95 A	16.9 ±0.73 B	0.0001 **
Suing liqnids mercy CB	1.4 ±0.06 D	8.7 ±0.52 C	35.1 ±1.83 B	46.3 ±2.35 A	0.0001 **
The averages that carry different letters with in the same row differ significantly between them **P≤ (0.01)					

which is found in the relationship between cytology and bacteriology with vaginal discharge scour. the study result that there was significantly increased neutrophil proportion rat (46.3± 2.35) in group of cows infected with EM2 in uterine fluid collected by cytobrush. This agrees with the fond of [4] Mammalian neutrophils are main source of oxidants in mammalian species as they produce a great amount of superoxide , hydrogen peroxide , HOCL, and other ros. the presence of neutrophils. are always consequences of inflammation. [10] the production of pro-oxidant species always start locally near the sites of tissue damage or infection but can become chronicle if the inflammatory response is not properly controlled . oxidative stress may negatively affect the neutrophil regulatory mechanism . and modulatory of neutrophil death that crucial for the evolution of the inflammatory process [8]. neutrophil cytolysis likely implies the amplification of inflammatory response and the accumulation of oxidation product (AOPP) at the inflammatory. site(uterus) may lead to the progressive reduction of neutrophil viability and may regulatory properties .Neutrophils contribute to the regulation the release of anti-inflammatory cytokine IL-LO [27] interestingly high levels of IL-LO were observed in the cows with subclinical endometritis which might contribute to the weakling of uterine resistance to pathogens and lead to the persistence of inflammatory postpartum [28]. According to the above the high plasma AOPP concentration observed in the EM2 in this study depend on general inflammatory status that activates peripheral neutrophils , and may well be an example of ox inflammation which if not adequately hindered by the animals homeostatic system could result in low fertility later during the postpartum.AOPP are considered as biomarker of neutrophil Ros generation [29].

The study concluded that oxidative stress and protein oxidation have an effect on dairy cow infertility by decreasing rate and early embryonic loss and having an effect on milk production parameters. The study also concluded that the inflammatory biomarkers (AOPP) are helpful in indicating the infected animal with subclinical en-

dometritis, which has no clinical signs at a time that leads to a decrease in the reproductive parameters.

References

1)Galvao KN , Santos NR, Galvao JS, and Gilbert RO,.(2011). Association between endometritis and endometrial cytokine expression in postpartum Holstein cows. Theriogenology . 76: 290- 299. Dol : 10.1016/j . Theriogenology., 02.006.

2)Opsomer . ; (2015) . Metritis and endometritis in high yielding dairy cows. Rev.Bras Reprod . Animal, Belo Horizont V.34,P.164- 172.

3)Salah N:and Yimer, N:(2017). Cytological endometritis and its agrcemenl with ultrasound examination in postpartum beel cows vet world to.

4)Gabai , Elisa betta, Giovanni ,Gianni Zin ,Annalisa ,and Lara etc.(2019) . Relationship between P. oxidant Biomarker and uterine healthy in dairy cows during P.P period . antioxidant , MDpl 8 – 21.

5)McGrath , J; Duval, S. M; Tamasssia , L. F. M; Kindermann , M; stemmler , R. ; T ;(2018). de Gouvea , V. N; Acedo , TS; Immig I; Williams , and S.N; Celi, P. Nutritional strategies in ruminants A life time approach , Res . Vet . Sci., 116,28 - 39 (cross Ref).

6)Talukder ,S; Kerrisk , K. L; Gabai , and celi , P.(2017). Role of oxidant – antioxidant balance in reproduction of domestic animals . Amin . Prod . Sci ., 57, 1588-1597.(cross Ref).

7)Celi, P.; Merlo , M.; Barbato, O,; and Gabai , G.(2012) Relationship between oxidative stress and the success of artificial insemination in dairy cows in a pasture – based system . Vet .j., 193,498-502.[Cross Ref].

8)Winterbourne,C .C.; Kettle , A.J.; and Hampton , M.B.(2016). Reactive oxygen species and neutrophil function Annu Reo Biochem .,85,765- 792. [Cross Ref].

9)Valacchi, G.; Virgili , F.; Cervellati , C.; and Pecorelli , A.(2018). Oxlnflammation form subclinical condition to pathological biomarker , front . physiolog , 9, 858. [Cross Ref] [Pub med].

10) Celi ,P.;and Gabai , G .(2015). Oxidant / antioxidant balance in animal nutrition and health :the role of protein oxidation .front .Vet . Sci., 2, 48 .[Cross Ref].[Pub-Med].

11) Kaufimann TB,Drdlich M,Tenhagen BA, Forderung D,and Heunieser W. (2008).Pervaknce of bovine subclinical endometritis 4h after insemination and its effects on first service conception rate .Theriogenology 71(2) : 385-391. doi: 10: 1016 / J Theriogenology. 08.005.

12) Bondurant,R.H.(1999).Inflammation in the bovine female reproductive tract . Journal of Animal Science ,77,101.

13) Singh I.Murray RD.Mshelia G, and Woldehiwet Z.(2007). The immune status. of the bovine uterus during the peripartum period . Vet J 175(3) :301-309.dol 10.1016/j.

14) Witko- Sarsat V, Friediander M, Capeillere – Blandin C, Nguyen –Khoa T, Naguyen A T, Zingraff J, Jungers p and Descamps- Latscha B.(1996)Advanced oxidation protein products as a novel maker of oxidative stress in uremia. Kidney Int 49: 1304-1313, 1996.

15) Senosy, ws ,uchiza , m-tamcoka, n-lzaike , y, and t,.(2009).ascociationbetween evaluation of the repondnctive tract by various diagnostic tested and restoration of ovarian cyclking in high – producing dairy cows theriogenology 72, 1153-1162, .

16) Sheldon IM, Lewis GS. LeBlane S,and Gilbert RO.(2006). Defining postpartum uterine disease in cattle .Theriogenology: 65: 1516- 1530.

17) Kasimanickam, R., Duffield , T, F, Foster , R. A, Gartley ,C, J., Leslic , K, E, Walton , J.S., and Johnson , W. H.;(2005). A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. Can . Vel. J., 46. 255-259.

18) Senosy ,w .uchiza, m, tamcoka,n, tzaika, y- and osaws , T.(2 011). Impact of ovarian and uterian conditiumon diagnostic test output of endometritis in post-partum lugs yielding dariy cows .repcod dom anim, 46,800 ,.

19) Ahmadi MR.dehgan sa, evaluation of the treatment of repeat breath breeder dairy cows with uterine lavage plus pgf with or without cepphapirin . Turkish Journal of Veterinary and Animal Sciences . 2007; 31: 125. 129.

20) Duncan , D. B . (1955) **.** Multiple Range Test and Multiple F.Test .Biometrics. 11.1-42.

21)SAS. (2012). Statistical Analysis System. User´s Guide. Statistical Version 9.1th. ed. SAS. Inst. Inc., Cary, NC. USA.

22) Al-Mohammed T .M. , A .H Mohammed , and Al-Shaty , E.R.(2013). Clinical , hematological and therapeutically study of puerperium merits in Holstein – Friesian cows . journal of kerbala university ,vol . 11 no .1 scientific.

23) Sheldon .Im; Cronin , J; Goetze , L ; Donofrio , G; and Schuberth , HJ,(2009). Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle . Biol. Reprod . 2009. 81, 1025- 1032 cross-ref) (Pubmed).

24) McDougall , S.; Hussein , H ,; Aberdein D,;and Buckle ,K .;(2011). Roche ,J,; Burke, C .; Mitchell ,M,; Meier , S . Relationships between cytology bacteriology and

vaginal discharge scores and reproductive performance in dairy cattle . Theriogenology.,76, 229- 240 [Cross Ref].

25) Peter, S; Michel, G.; Hahn A.; lbrahim , M.; Lubke –Becker ,A.;Jung , M .; Einspanier , R,; and Gabler,C.(2015). Puerperal influence of bovine uterine health status on the mRNA expression of pro- inflammatory factors J. Physiol . Pharmacol ., 66, 449- 462.

26) Celi, P.; Merlo , M.; Da Dalt, L.; Stefani , A.; Barbato, O,; and Gabai , G.(2011). Relationship between late embryonic mortality and the increase in plasma advanced oxidized protein (AOPP) in dairy cows . Repro'd . Fertile Deo . 23, 527-533.

27) Christoffersson , G.; and Phillipson,M.(2018) . The neutrophil : one cell on many missions or many cells with different agendas ? cell tissue res., 371, 415- 423. .2013, 96, 4333-4339. [Cross Ref] [Pub Med].

28) Brodzki , P; Kostro , K; Brodzki , A: Wawron , W; Marczuk, J; and Kurek , L (2015). Inflammatory cytokines and a cute phase protein concentrations in the peripheral blood and uterus of cows that developed endometritis during early postpartum . theriogenology., 84, 11-18(cross Ref) (PubMed).

29) Winterborne,C.C.(2002). Biological reactivity and biomarkers of the neutrophil oxidant. [Cross Ref].Hypochlorous acid . toxicology, 181- 182, 233- 227.