The study of relationship of Advanced Oxidation protein products and some hormones with uterine disturbances

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Abstract
The study was conducted on 100 of Dairy cows in Tag-Alnahrain station sited in Al- Qadysia province post parturition (after 40-60) day after calving. The aim of study was to test the relationship between oxidative stress particularly protein oxidation and uterine health during the puerperium. The cows classified into four groups according to intense of clinical signs of merits. and neutrophil percentage in uterus and vaginal mucus discharge, in this study the cut off value of PMN %.was set at 8% to distinguish between degrees of inflammation.the group (H) was (54). healthy cows,(SCE) was 22 cows infected with subclinical endometritis, (EM1) was (11) cows infected with clinical endometritis Grad 1 and (EM2) group was (13) cows infected with clinical endometriosis Grad 2 the Advanced oxidation protein product (AOPP) was estimated in plasma and vaginal mucus discharges by using commercial laboratory method to detect the dityrosin formation which is the major content of AOPP. AOPP the good marker of protein oxidation were visualize by western blot (BCA Protein Assay Kit; Pierce Biotechnology, Rockford, LL, USA) analysis (Witko- sarsat etal, 1996). the study demonstrate that there was increasing in plasma AOPP in EM2 group in significant value of p≤0.01, Also the study show significantly increase in the concentration of AOPP in vaginal mucus discharges in group of animal infected with (SCE) the study clarified that there was increasing in neutrophil pointage over than 8% in vaginal mucus discharge after collecting mucus by metrecheek in(EM1) group in significant value of p≤0.0l while it was high significantly(p≤0.01) in uterine mucus collected by cytobrush. The study proved positive correlation between AOPP concentration and progesterone while its correlate negatively with estrogen both effect impaired fertility by increasing the interval period and repeat breeder leading to increase the number of serves to and also the open day of dairy cattle infected by SCE in significant value of p≤0.01 the study clarified that there was obvious effect for merits on decreasing milk production period and the mean of milk product/day and total of milk product in lactation period this decline was higher significantly p<0.01 in dairy cows with high infected with merits. the study proved that oxidative stress and protein oxidation antigenic effect of AOPP in uterine was a reason and biomarker impaired fertility in dairy cow infected with merits.
keyword: protein oxidation, merits, sub clinical merits, oxidative stress.

1. Introduction

The main characteristic of cow production is reproduction, because many cows do not achieve peak reproductive performance thus result in high significant Loss (Galvao etal., 2011). studies show that 75% of postpartum disturbances in cattle occur during the first 30 days after parturition.like disease and uterine bacterial infection .(Gabai etal.,2019).which impair postpartum immune activity,Hormonal and metabolic functions and energy balance. (Opsomer etal.,2015). Physiologically the composition of uterine micro flora change over time and bacteria cleared,However the risk of developing clinical merits remain elevated especially in cows that deliver twins or experience still birth, dystocia, retained placenta or metabolic disorders (Salah,2017) more over the development of subclinical endometritis can impact negatively on fertility.(Gabai etal.,2019) the present of high neutrophil counts in the endometrium 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 .(McGrath etal.,2018). in dairy cow both oxidant and antiox involved in regulation of follicular fluid environment, follicular genesis, steroidegeness, corpus Lutein function and lutolysis (Talukder etal.,2017). in dairy cows oxidative stress and protein oxidation are implicated in the embryonic death (Celi etal .,2012). neutrophils are the major source of oxidant in dairy cows they produce the enzyme NADPH oxidase that once activated generates amount of superoxide o^{-2} which is precursor of 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 to reacting with halide and giving highly microbiocidal species (hypochlorous acid) (HOCL) which is a

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potent bactericide molecule that can also modify extracellular target and effect the function of neighboring cell (Winterbourne etal.,2016).(Hocl) attacks proteins inducing alteration to protein backbone and Sid chains. so it alters the protein function as receptors enzymes, transport or structural proteins and generate new antigen (Celi etal., 2015). The products of protein side – chain oxidation are preferable to assay because of their stable state and easy to detected them. The most frequently used biomarker of protein oxidation is advanced oxidation protein product (AOPP) and may be considered the more stable marker of oxidative stress linked to phagocytes activity .(Valacchi etal., 2018) plasma profiles of the (AOPP) Albumin ratio provide amost sensitive indication of protein oxidation.(Celi & Gabai ,2015) in dairy cows pathogens are often introduced in uterus during the (AI) procedure and generate an inflammatory reaction leading to protein oxidation and (AOPP) generation ,the development of subclinical endometritis to be a common event in dairy cows after artificial insemination .(Kaufmann etal .,2008). so when uterine physical defenses are breached the next line of defense is represented by neutrophils triggering an inflammatory process the resulting activated Leukocyte and vasoactive substances released during. Inflammation can increase blood vessel permeability resulting in plasma protein Leaking into the endomaterial surface (Bondurant, 1999), (Singh etal ,2007). so the aimed of this study is to assay the (AOPP) as inflammatory marker of protein oxidation in dairy cow affected by different stage of merits to understand the role of protein oxidation in the pathophysiology of reproduction wastage.

2. Material and Methods

Animal of study

The study have been conducted on (100) of freezing – holes tin dairy cows in TaG Al – nahrain farm located in Al-kadesia provine – Iraq for period from march 2021 to march 2022. They received reproductive management by clinical check-up performed soon after calving (20-40 day) though the reproductive information were collected from parturition and health records saved in the station. All of the cows suffer from uterine disorder after calving so they divided in to four groups (H ,SCE, EM1, EM2) according to mucous vaginal scour and uterine cytology and the aid of veterinary team working in the farm.

Clinical Tests

Data were collected form the farm records contained number ,Age, calving number, times of abortion, oestrus regulation ,(Al) system, the number of conceives ,the type and time of last calving and reproductive problem and disease infected with.

Samples Collection

Blood samples (5-10)ml were aspirated collected from the tail vein using evacuated EDTA tubes for plasma (AOPP) analysis by the manner of (witko - sarsat ,1996).by using kit (BT, Lab, animal advanced oxidation product ELISA kit.(Gabai etal.,2019) another blood sample were collected in evacuated without EDTA tub for serum hormone concentration measure.

A-progesterone measure by ELIZA kit .(Accubind kit usp).(Gautray ,1981).

B- estrogen measure by ELIZA kit. (Accubind kit usp).(Barnett, 1973).

mucous secretion collected

evaluation of vaginal mucous

Vaginal Mucus was collected similar to manner described with (senosy etal.,2009) who conducted after cytobrush assessment by inserting the metercheck device into vagina. the cup of device insorted into the external uterine orifice. then the opposite side. of the device elevated slightly to fill the cup with vaginal mucus. the device was pulled gently from the vaginal mucus. was scored according to. study of (Sheldon etal.,2006) and(senosy etal., 2009).On a (0 to 3) scale (score 0 represents clear mucous ; score 1 represents mucus containing flucks of white or off- white PUS ; Score 2 represent mucus discharge contain < 50% white or muco-purulent material ; and score 3 represents discharge contain ≥50% purulent material white or yellow but sometimes sanguineous).

Evaluation of endometrial cytology

Endometrial cytology was performed by cytobrush according to studies of (Kasimanickam etal .,2005; Senosy etal .,2011) before the use of cytobrush was sterilized with formaldehyde gas. the external organs of reproductive and their margined where cleaned with benzalkumium and alcohol before the cytobrush inserted in to the vagina. when the cytobrush was passed the cervix and inserted in to the base of uterine body under rectal operation .the brush was exposed on the dorsal membrane of the uterine wall .then the endometrial samples collected by rotating (36^0c)twice while in contact with dorsal wall.

Cytology slides were prepared by rolling the cytobrnsh on three clean glass slides and immediately fixed with cytokeeep (Nippon shoji, Oska, Japan). the slides were stained with giemsa stain with in 2h after preparation. to assess endometrial inflammation .by PMN in an objective way cytological assessment was determined by counting a minimum of (200) cells at (400x) magnification (Ahmedi etal., 2007; Senosy etal., 2009). the threshold cutoff of PMN% was conducted on PMN≥ 8% for normal PMN < 8% for inflammation.

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 Duncan test (1955) and by using SAS program (2012).

3. 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 (Al-Mohamed etal.,2013).

Tab1: 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%.

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

the study result by Bacteriological test showed that each cows was infected with at Least one or more genius or species of Bacteria in uterine and vaginal fluid as it show in Tab (2). This is improve that the Bacterial contamination was occurred in most animal of study and there was no relationship between Bacterial merits and protein oxidation biomarker (AOPP) though the cow uterine usually contaminate with multikind of Bacteria but don't develop to clinical merits (Sheldon etal.,2009) while cows bacterial infect specially with E.Coli and pervotel pyogenic result from bacterial isolation was mor pathogenic type. found in 8 cows of EM2 group this result was in consist with the found by (McDougll etal .,2011) and (Peter etal., 2015).

Tab2: cows positive for bacterial species and cultivars isolated by bacteriological culture from uterine and vaginal swab.

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

the study demonstrate that there was significantly increasing (p<0.01) in plasma AOPP of group (EM2) show in tab(3) this result agree with that found by (Celi & Gabai, 2015 and Celi etal.,2011) so the study result that there was significantly increasing of AOPP p<0.01 in vaginal mucus of SCE groups of cows which was do not demonstrate any clinical sigh of merits and had mucus score more than 2 and PMN % more than threshold cut of PMN ≥ 8% this improve that the AOPP is best biomarker for SCE that always cause repeat breeder and failure ovulation also effect on reproductive performance. the AOPP was increased as result of neutrophil activation during uterine infection early postpartum or once through the first conception and represent useful markers of MPO/ HOCL protein oxidation. (Celli etal ., 2011).

Tab3: The concentrations of AOPP in blood plasma and vaginal mucus.

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

the study show that there was significantly increase (p<0.01) in neutrophil proportion (22.3± 0.95) in (EM1) animal group shows in Tab4. in mucous secretions collected by met check devise this result agree with the found of (McDougall etal ., 2011).

Tab4: the percentage of neutrophils in uterine fluids and vaginal mucus using metrcheck device and cytobrush.

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

which is found the relationship between cytology and bacteriology with vaginal discharge scour. the study result that there was significantly increasing in neutrophil proportion rat (46.3± 2.35) in group of cows infected with EM2 in uterine fluid collecting by cytobrush this agree with the fond of (Gabai etal., 2019) neutrophils are main source of oxidants in mamma line species as they result great amount of superoxide, hydrogen peroxide ,HOCL and other Ros. the present of neutrophils. are always consequences of inflammation.(Valacchi etal., 2018) the production of pro-oxidant species always start locally near the sites of tissues damage or infection but can become chronicle if the inflammatory response is not properly controlled. oxidative stress may negatively effect the neutrophils regulatory mechanism. and modulatory of neutrophil death that crucial for the evolution inflammatory process.(Winterbourne etal.,2016) 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 .neutrophil contribute to the regulation the release of anti-inflammatory cytokine IL-10 (Christoffersson etal.,2018) interestingly high levels of IL-10 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 (Brodzki etal.,2015). 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 anexample 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 reactive oxygen species reactive oxygen species (Ros) generation.(Winterbourne,2002).the result show in Tab (5)sever hormonal disturbance in both estrogen and progesterone during postpartum the result. clarified that the progesterone concentration in serum of animal increase significantly p<0.01 according to intensity of grad of inflammation so it reached (6.4± 0.9) ng / ml in Em2 animals. the metritis effect cows fertility by two side the first, in cows the uterine injury by destruction of uterin endometrium tissue by accumulation of AOPP.(resedue of protein oxidation) the other side by bacterial endotoxin effect the release of progesterone & estrogen by alteration the follicular growth and normal growth of corpus leutume (Williams etal.,2007) also initiated with LH function for induced the ovulation result in it's failure (Lavon etal.,2010).also they induce the release alarg amout of PGE from uterine endometrium causing the prolonged of corpus luteum life span (Herath etal.,2009). this was obvious in the result show in Tab 5 there was increase of progesterone concentration these results agree with the found of (Gobikrushanth etal.,2016) in the clinical endometritis grad 1 and grad 2 could be pathogenic isolated some of bacteria like E.coli and prevotella pyogene (Pruner etal.,2014) but in subclinical endometritis no pathogenic bacteria could be isolated there was only total of neutrophils remain in the uterus after bacteria elemenation to keep the inflammation and there was imbalance between pro-inflammatory cytokine and anti-inflammatory cytokine during the first weeks after calving play regressive role in the sequence of subclinical metris specially function of ovary like follicular growth regulation, corpus lutein formation and inflammatory media regulation,(Field etal.,2013) beside that there was accumulation of reactive oxygen species (Ros) in the uterine cell and follicular cells this lead to apoptosis of this cell and loss function and so on effect the estrogen formation and its concentration decrease in serum according to intense of inflammation as it show in Tab(5) was reached to 210.42±1.31.

Tab5: estrogen and progesterone levels with uterine inflammation classes and the correlation coefficient between them.

Estrogen Correlation Ml Ng	Progesterone Correlation Ng /Ml	Clinical Classificatis of Animles
1.5 A±288.21	1.9 C±2.6	H
1.4 B ±220.13	1.6 B±5.2	SC
1.5 BC ±215.41	0.20 B ±6.9	EM1
1.31 C ±210.42	0.9 A ±10.4	EM2
0.0027 **	0.0001 **	Morale leved
The averages that carry different letters with in the same column Differ significantly between them)P≤0.01.(		

the result significante at level of 0.002 this result improved the significant negative correlation between estrogen (E2) and AOPP (-0.51) shows in Tab 6 while the correlation between (p4) progesterone and AOPP was significantly posetive (0.86) at level of p<0.01 as it show in Tab 6.

Tab6: correlation coefficient between AOPP and E2 and P4 in cases of uterine inflammation.

Morale leved	Correction coefficient (r)	Adjectives
*	-0.51	AOPP و E2
**	0.86 **	AOPP و P4
*	-0.65 *	E2 و P4
) *P≤0.05) ** ،(P≤0.01.(		

this result explain the effect of AOPP on regulation of corpus lutein growth and prolonged of its life span (Sakumoto etal ., 2003).

the study results in tab(7) improved the effects of merits on reprod active performance in each type but the major effect was in SCE group of cow which demonstrated inereasing in the interval period (580 ± 3.7)open days (205±1.92)and the number of services per conception (5.5± 0.26)this difference was significant at p≤0.01, this result was agree with what found by (Leblance etal .,2014, Eckel& Amentaj,2016) the effect of SCE on cows envolved by total of neutrophil the major sours of Ros which lead to oxidative. stress especially protein oxidation causing disturbance of ovary and uterine function, this result confirmed by previous studies (Gilbert etal., 2005, Plontzke etal 2010, Barlund etal., 2008) despite the difference in diagnoses methods and the period after calving and PMN% threshould cut off in addition to other factor like estrus detection failure, insufficient nutrition, lack of health care and the cows getting old bad in insemination and other disease.

Tab7: the effect of uterine inflammation on reproductive efficiency indicatory.

Interval period /Day	Number of Conceive	Open deys	Clinical Classificatis
2.5 A ±350	0.63 A ±1.51	4.3 A ± 166	H
3.7 C ±530	0.26 C± 5.5	1.92 B ±205	SC
4.64 B ±475	0.17 B ±3.5	12.6 A ±175	EM1
3.4 B ±478	0.25 B± 3.1	18.4 A ± 176	EM2
0.0047 **	0.0001 **	0.0061 **	Morale leved

The averages that carry different letters with in the same column Differ significantly between them) **.P≤0.01.(

the result of study in showed that there was significant decrease in milk production parameter like period of lactation season (180.6± 3.4, 160.4± 3.5, 167± 3.9) days mean of milk production /day (5.86 ± 1.13,4.7 ±1.13,4.31 ± 1.8)kg /day and the total of milk production/season.(1058.31±1.34, 779.24±11.7,719.77±11.5)kg /season according to the intensive of infection and grad of merits. the differences was significant at (p<0.01) the study clarified that the infection with meteorites effect on each milk production parameter. it was shown by measuring the plasmatic AOPP in cows infected with subclinical and clinical merits there was decreasing in milk production parameter compared with healthy cow. that was indicator to the involvement of oxidative stress and oxidizing protein in mammary gland function and their immunity. this was confirm by (Guzzo etal ., 2015 and Gabai etal., 2014) who suggested the plasma AOPP decrease in period around parturition and increase post parturient because of exposing to inflammatory stat this result is agree with what found by (Bell etal., 2007) and (Dubuc etal.,2011) who referred to decrease of milk production in dairy cows with infected clinical and subclinical merits and suffer of bad management tab 8.

Tab8: the effect of uterine inflammation on milk production and the length of the production season.

Long of milk Product Period	Mean of milk Product /day Day /kg	Total of milk Product	Clinical Classification
10.33a ±200.12	1.2 a ±8. 21	20.7 a ±1642.98	H
3.4 ab ± 180.6	1.13 b ± 5.86	13.4 b ±1058.31	SC
3.5 b± 165.4	1.13 c ± 4.71	11.7 c±24. 779	EM1
3.9 b ±167	1.8 c ±4.31	11.5 c ±719.77	EM2
0.0001 **	0.0001 **	0.0001 **	Morale level

The averages that carry different letters with in the same column Differ significantly between them)**P≤0.01.(

4. Conclusions

the study concluded that oxidative stress and protein oxidation effect on dairy cows infertility by decreasing pregnancy rate and early embryonic loss and effect on milk production parameter also the study concluded that the inflammatory biomarker (AOPP) are helpful in indication the infected animal with subclinical endometritis which have no clinical signs at a time that lead to a decrease in the reproductive parameters.

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