RELATIONSHIP BETWEEN OXIDATIVE AND CYTOGENETIC STATUS OF DAIRY COWS AND RECURRENT SUBCLINICAL MASTITIS CAUSED BY *STAPHYLOCOCCUS AUREUS*

EBTIHAL A. IBRAHIM[*], MAHA A. IBRAHIM[*] and EBTSAM E.Z. KOTB[**]
[*] Department of Biology of Reproduction, Animal Reproduction Research Institute, Alharm- Giza, Egypt.
[**]Department of Mastitis and Neonatal Diseases, Animal Reproduction Research Institute, Alharm-Giza, Egypt.

ABSTRACT

Received at: 24/3/2012

Accepted: 30/4/2012

Dairy cattle are susceptible to a variety of metabolic and infectious diseases. Mastitis is one of the most important diseases in dairy production sector causing losses in milk production. Concerning the subclinical mastitis (SCM), the main form of mastitis in modern herds, Staphylococcus *aureus (S.aureus)* is the most frequent and major pathogen causing it. So, the current work was conducted to determine the possible relationship between the recurrent SCM caused by *S.aureus* and the changes that occur in the oxidant / antioxidant parameters and the cytogenetic picture of cows using these changes as predictive biomarkers. Ten healthy and 21 recurrent SCM cows infected with *S.aureus* were selected after bacteriological examination. Milk and 2 blood samples were taken from each cow. Milk sample was used for California Mastitis Test (CMT), Somatic Cell Count (SCC) and bacteriological examination. First blood sample was used for plasma separation and estimation of malondialdehyde (MDA), Nitric Oxide (NO), Total Antioxidant Capacity (TAC), glutathione peroxidase (GSH-Px) and catalase (CAT). The second whole blood sample was used for cytogenetic examination. Results of SCM cows revealed imbalance between the oxidant (MDA and NO) and antioxidant (TAC, GSH-P_X and CAT activities) parameters. Additionally, cytogenetic changes were represented by increasing the percentage of gaps, deletions and total aberrated cells. In conclusion, the recurrent SCM cows affected with *S.aureus* were undergoing the stress of lactation, infection and inflammation that consequently alter the oxidative status and cytogenetic picture of affected cows.

العلاقة بين حالة الأكسدة والتغيرات الوراثية الخلوية فى الإبقار الحلابة والتهاب الضرع المتكرر بدون أعراض مرضية والمسبب بميكروب العنقودي الذهبي

ابتهال إبراهيم ، مها إبراهيم ، ابتسام السيد زكى قطب

تعتبر الأبقار الحلابة هى الأكثر عرضة لأمراض الأيض ومختلف الأمراض المعدية. والتهاب الضرع هو أحد أهم أمراض قطاع الإنتاج الحيواني والذي يسبب خسائر اقتصادية فادحة نتيجة نقص إنتاج اللبن. والتهاب الضرع الغير مصحوب بعلامات مرضية هو الأكثر شيوعاً فى المزارع الحديثة. وقد وجد أن الميكروب العنقودي الذهبي هو سبب رئيسي لحدوث وتكرار مثل هذه الإصابة. وقد أجريت هذه الدراسة بهدف تحديد العلاقة المحتمل وجودها بين مرض

التهاب الضرع المتكرر الغير مصحوب بأعراض مرضية ظاهرة المسبب ميكروب العنقودي الذهبي والتغيرات التي يمكن أن تحدث فى مستوى كل من المؤكسدات ومضادات الأكسدة بالإضافة إلى التغيرات الكروموسومية الحادثة والتي تعتبر علامات حيوية قد تفيد فى التنبؤ والتشخيص المبكر للمرض لتلافي تكرار حدوثه. وبناءً على تاريخ الحيوانات المرضى المدون بالسجلات ونتائج الفحص البكتيري فقد تم اختيار ١٠ أبقار سليمة صحياً وتم اعتبارها المجموعة الضابطة، أما المجموعة الثانية فقد تكونت من ٢١ بقرة تاريخها المرضى يفيد اصابتها بالتهاب الضرع المتكرر والذى أثبت الفحص البكتيري اصابتها بالميكروب العنقودي الذهبي. وقد تم تجميع عينات لبن لعمل اختبار كاليفورنيا وعد الخلايا الجسمية SCC بالاضافة الفحص البكتيري. أما عينات الدم فقد تم سحب عينتين للدم الأولى لفصل البلازما واستخدمت فى قياس مستوى كل من المالوند الدهيد، أكسيد النيتريك، مضادات الأكسدة الكلية، انزيم الجلوتاثيون بيروكسيديز وإنزيم الكاتاليز أما العينة الثانية فقد استخدمت كدم كامل للفحص الكروموسومي. وقد أظهرت نتائج الدراسة وجود زيادة معنوية فى مستوى المؤكسدات بالدم والممثلة فى زيادة المالونداهيد وأكسيد النيتريك وفى المقابل كان هناك نقصاً معنوياً فى مستوى مضادات الأكسدة والمتمثلة فى مضادات الاكسدة الكلية وتناقص نشاط إنزيم الجلوتاثيون بيروكسيديز وانزيم الكاتاليز. أما بالنسبة للتحليل الكروموسومى فقد كان هناك زيادة معنوية فى النسبة المئوية للاختلالات التركيبية الكلية بالاضافة إلى النسبة المئوية للأجزاء الكروماتيدية الممحاة والفجوات أما الاختلالات العددية والتركيبية الاخرى فقد سجلت زيادة غير معنوية احصائياً. ونستخلص من هذه الدراسة أن الأبقار الحلابة تقع تحت تأثير أكثر من عامل من عوامل الاجهاد منها فترة الحلب نفسها وزيادة احتياجات الجسم فى هذه الفترة بالإضافة إلى الاصابة البكتيرية والالتهاب الناتج عنها، والذى يؤدى إلى الخلل بمستوى المؤكسدات ومضادات الاكسدة فى الجسم علاوة على التغيرات الكرموسومية الحادثة. وهذه التغيرات يمكن استخدامها كعلامات حيوية للتشخيص المبكر للمرض والتنبؤ بوجود الميكروب العنقودى الذهبى الكامن بالضرع فى محاولة للتقليل من خسائره الاقتصادية.

Key words: *Bovine recurrent subclinical mastitis, S.aureus, oxidative stress, cytogenetic changes.*

INTRODUCTION

Bovine mastitis is one of the most important production diseases of dairy animals which is directly or indirectly affect the farmers and ultimately affect the economy of the country. However, mastitis is a global problem as it adversely affects animal health, quality of milk and economic of milk production (Sharma *et al*., 2012b). Since mastitis is a diseases caused by multiple factors (multiple pathogens), it is difficult to control (Andrei *et al*., 2011). So, solutions leading to reduction in the incidence of mastitis are highly demanded (Sodeland *et al*., 2011). Regarding subclinical mastitis (SCM) the main form of mastitis in modern dairy herds (Zhao and Lacasse, 2008), it is considered as the most economically important type of mastitis (Karyak *et al*., 2011). Its cost is very difficult to quantify but causes loses more than dose clinical mastitis. Moreover, approximately 70% of these costs are associated with reduction in milk production, 9% milk discard after treatment, 7% cost of veterinary services and 14% premature culling (Zhao and Lacasse, 2008; Sharma *et al*., 2012a). Sub clinical mastitis is subtle and more difficult to detected as the cow, the udder and the milk seems normal (Bhupal, 2007). Meanwhile, microorganisms and white blood cells (somatic cells) that fight infections are found in elevated numbers in milk. Additionally, most clinical cases start as subclinical; so that, controlling SCM is the best way to reduce the clinical cases (Andrei *et al*., 2011). Ferthermore, More than 200 infections causes of bovine mastitis are known. The commonest pathogens are *staphylococcus aureus (S. aureus), streptococcus agalactiea, other streptococcus* and *coliforms* (Yong *et al*., 2009; Sharma and Maiti, 2010). *S. aureus* has emerged as one of the most prevalent and predominant contagious mastitis causing pathogens that colonize the teats when there is damage to the skin surface (Abdel Hameed *et al*., 2008; Yang *et al*., 2011b). Most often infections caused by *S.aureus* are subclinical in nature with periodic flare-up of clinical symptoms (Bramely and Dodd, 1984). The high prevalence of *S.aureus* is mainly attributed to the wide distribution of microorganism inside the mammary gland and on the skin of teats and udder (Yang *et al*., 2011a) while it survives outside the cow for a short time only (Risco *et al*., 1999). During inflammatory disease status, immune cells produce Reactive Oxygen

Species (ROS) (Sordillo and Aitken, 2009) which become harmful to immune cell itself and can decrease the ability of the immune system to respond to infection (Spears and Weiss, 2008). The unstable compounds of ROS (e.g. malondiadehyde- MDA and Nitric Oxide NO) interact with lipids, proteins, DNA and other biomolecules within the body to induce instability and create tissue damage (Zhao and Lacasse, 2008; Yang *et al*., 2011b). Oxidative Stress (OS) induced by ROS is believed to be a primary factor in various cattle diseases including mastitis (Karyak *et al*., 2011). Oxidative stress increase causes a continuous increase in the concentration of lipid peroxidation products (MDA) and decrease in level of enzymatic and non-enzymatic antioxidants after transiently increased activity to combat the toxic effect of ROS (Sharma *et al*., 2011).

So, the objective of this study was to explore the possible interrelation between the recurrency of SCM caused by *S.aureus* and oxidative and cytogenetic status of affected cows and also to predict the recurrency of SCM by measuring of some oxidant (MDA and NO) / antioxidant (TAC, GSH-Px and CAT) parameters and cytogenetic changes as predictive biomarkers.

MATERIALS and METHODS

Animals:

The current study was done in a private farm in Alexandria governorate on 61 dairy Holstein cows. All cows were apparently healthy, their age ranged from 5-7 years. All animals were subjected to California Mastitis test (CMT), somatic cell count (SCC) and bacteriological isolation and identification to determine the causative agent of mastitis. The main problem of these animals was the recurrency of mastitis. Ten cows were negative CMT, normal SCC and negative bacteriological examination and these were the control healthy group. Twenty one recurrent SCM cows infected with *S.aureus* microorganism as a single infection were selected from all cows under investigation after bacteriological examination and considered as SCM group.

Samples:

1- Milk samples: Milk samples were collected from each cow before morning milking. No cow had any evidence of clinical mastitis. After teat cleaning (with water then 70% ethanol), first streams of milk were discarded and then about 10 ml of milk was collected aseptically into sterile screw capped McCartney bottles. The samples were stored in ice box and transported to the laboratory for examination within two hours after collection.

2- Blood samples:

Two blood samples were collected from each cow by jugular vein puncture. The first sample was taken in heparinized vacuum tubes for plasma separation and estimation of oxidant/ antioxidant parameters (malondialdehyde- MDA, Nitric Oxide- NO, total antioxidant capacity- TAC, glutathione peroxidase – GSH-Px and catalaze- CAT). The second sample was taken in sterile heparinized vaccutainers for cytogenetic analysis.

SCC determination:

SCC was performed automatically using SOMA-COUNT 150 from Bentley (USA).
Milk samples were classified into 2 categories, normal (values below 200,000 cell / ml^{-1}) and subclinical mastitis (values above the limit of 200,000 cell / ml^{-1}) according to the National Mastitis Council (1999).

Bacteriological isolation and identification:

Bacteriological isolation and identification was done on specific media for *S.aureus* (sheep blood agar, manitol agar and brain-heart infusion agar), specific media for *Str. aga*. (Edward media), and specific media for *E-coli* and *coliforms* (MacConky agar) according to Toply and Welson (1998).

Biochemical examination:

Samples were examined using commercial diagnostic kits (bio-diagnostic) for the following parameters: MDA according to Ohkawa *et al*. (1979), TAC according to Koracevic and Koracevic (2001), GSH-Px

according to Paglia and Valentine (1967), and CAT according to Aebi (1983). NO level was measured using ELISA reader according to Rajaraman *et al*. (1998).

Cytogenetic analysis:

To 5ml of RPMI media 1640, in flattened side tubes, 1 ml of blood, 1ml fetal calf serum and 0.1 ml phytoheamaglutinine (PHA) were added. Then samples were incubated in CO_2 incubator at 37°C and 5% CO_2 for 72hrs. 0.01/ml colchicine was added to the samples one and half hour before the end of incubation period then reincubated again for one and half hour to complete the incubation period. After centrifugation (1000rpm/10min) and removal of the supernatant media, 0.56/ kcl were added to the samples and incubated for 30 min/ 37°C. Then the cells were fixed by carnnoys fixative (1part glaical acetic acid + 3 parts absolute methanol) for 3 to 4 times (centrifugation and removal of the supernatant in each time). Then the cell suspension was splashed on wet chilled slides then flamed to dry (Macgregor, 1993). Staining of the slides were done using 10% Giemsa stain and covered by DPX mounting media (Ram and Arvid, 1995). Each sample was scanned by examination of 50 good metaphase (Nicholas, 1996).

Statistical analysis:

All data's were analyzed by using student's T-test to know the significance values between groups. The percentages of chromosomal aberrations among groups were compared using chi-square test. All statistical parameters were calculated as per the Snedecor and Cochran (1982).

RESULTS

Somatic Cell Count

SCC of healthy group were ranged from/ 30.000-195.000 cell/ml with a mean of 149.500 cell / ml. While the range of the recurrent SCM group was ranged was ranged from 327.000- 764.000 cell / ml with a mean of 363.380 cell / ml.

Bacteriological examination:

As shown in Table 1 by using specific media for *S.aureus*, the present work recorded results reveal the prevalence of *S.aureus* pathogen was 34.43% as single isolate among all isolates.

Oxidant / antioxidant parameters:

Table 2 showed that the recurrent SCM cows affected with *S.aureus* pathogen have a significant ($P< 0.05$) increase in the levels of the oxidant parameters (malondialdehyde and nitric oxide). Mean while the same group recorded a significant ($P< 0.05$) decrease in the activities of antioxidant parameters (total antioxidant capacity, glutathione peroxides and catalase) compared with the healthy cows.

Cytogenetic examination:

Table 3 recorded the results of the numerical and structural chromosomal aberrations in all cows under investigation. Photo (1) showed normal metaphase spread 58 acrocentric autosomes and 2 submetacentric gonosomes (60, XX). The present results recorded a significant ($P< 0.05$) increase in the percent of cells showing gaps and deletions (photo2 &3) in addition to significant ($P<0.01$) increase in the total percent of aberrated cells. While the other numerical and structural aberrations recorded statistically non significant increase compared with healthy non mastitic cows.

Table 1: Prevalence of bacterial isolates.

Microorganisms	No.	Prevalence%
S.aureus	21	34.43
Coliforms	15	24.95
Str.aga.	14	22.95
Other Staph.	11	18.03
Total	61	100

Table 2: Oxidant/ antioxidant parameters in healthy and recurrent SCM cows affected with *S.aureus.*

Parameter	Healthy cows	SCM cows
MDA nmol/ml	2.01 ± 0.22	3.37 ± 0.4 **
NO nmol/ L	11.79 ± 0.86	18.94 ± 1.3**
TAC mmol/L	0.39 ± 0.04	0.22 ± 0.01 **
GSH- P_x mm/ml	25.35 ± 1.8	19.76 ± 0.56**
CAT u/ml	3.44 ± 0.07	2.65 ± 0.24*

* Means significant from healthy at (P< 0.05).

Table 3: Percent of Chromosomal aberrations in healthy and recurrent SCM cows affected with *S .aureus.*

% of chromosomal aberrations	Healthy cows	SCM cows
Peridiploidy	0.80	1.04
Breaks	0.40	0.57
Gaps	1.20	3.43*
Detetions	2.00	7.43*
Fragments	1.60	1.90
Total	6.00	14.37*

*Means significant from healthy group.

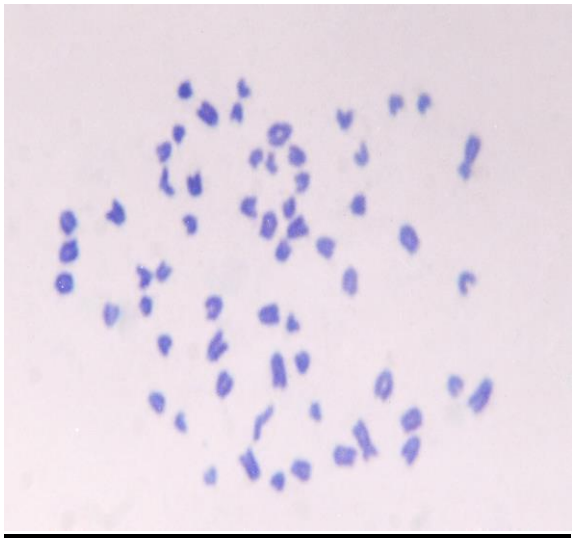

Photo 1: A normal metaphase spread (60-XX).

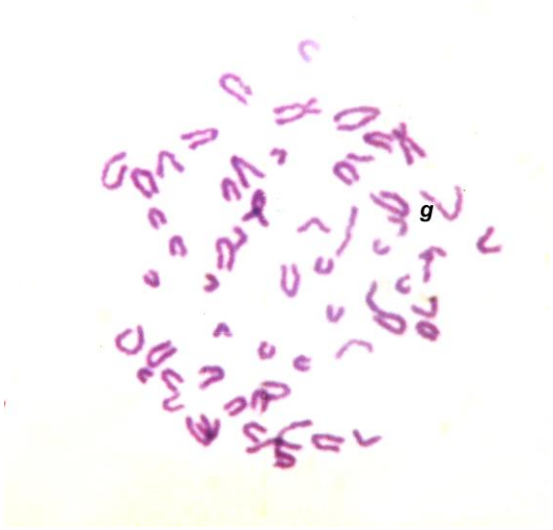

Photo 2: A metaphase spread showing gap (g).

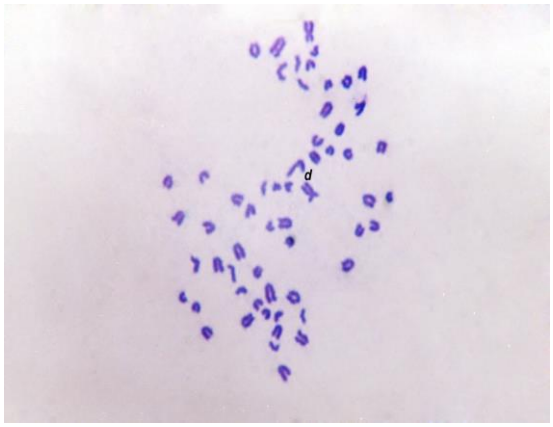

Photo 3: A metaphase spread showing deletion (d).

DISCUSSION

Dairy cows undergo massive metabolic adaptations during lactation. It was postulated that some of these physiological events may negatively impact the health of dairy cows (Sordillo *et al*., 2009; Sharma 2012 b). Consequently, those cows become more susceptible to a variety of metabolic and infectious diseases (Sordillo *et al*., 2007). They seemed to have more oxidative stress and low antioxidant defense mechanisms and this seemed to be probable reason for their increased susceptibility to production diseases (e.g. mastitis, metritis, retention of fetal membranes) and other health problems (Sordillo, 2005).

Subclinical mastitis (SCM) is considered as one of the most prevalent diseases in dairy cows, causing drastic loss in dairy industry (Karyak *et al*., 2011). *S.aureus* is the major pathogen causing it (Yang *et al*., 2011b). In the present study, *S.aureus* represents 34.42% as a single isolate from the total bacterial isolates of the recurrent SCM cows. There is a lack of studies that dialed with recurrent SCM caused by that pathogen. However, *S.aureus* is recognized worldwide as a frequent cause of subclinical intramammary infections in dairy cows

(Momtaz *et al*., 2010). Yang *et al*. (2011b) recorded that the common isolate from SCM cows were *S.aureus* as it represented 47% of the total isolates. They attributed the high prevalence of it to the wide distribution of microorganism inside the mammary gland and on the skin of teats and udder. Despite an apparently good antimicrobial susceptibility in *vitro*, the cure of diseased animals from this bacteriological infection is often disappointing, which results in cases of recurrent clinical and subclinical infections. The recurrency of *S.aureus* infection can be attributed to the growth of bacteria in biofilm so, it become highly resistant to antimicrobial agents (Melchior *et al*., 2006). Moreover, Momtaz *et al*. (2010) suggested that *S.aureus* produces a spectrum of extracellular protein toxins and virulence factors which are thought to contribute to the pathogenicity of the organism. So, fighting *S.aureus* in a herd requires a systemic program which can be summed up in sampling, culling, grouping and dry cow therapy (Ahlner, 2003).

Regarding to the oxidant /antioxidant status of SCM cows in the current work, it was revealed elevated levels of MDA and NO, on the other hand, decreased activities of TAC, GSH- Px and CAT. With respect to MDA, the lipid peroxidation end product, it is one of the most important consequences of oxidative stress indexes. The determination of lipid peroxidation allows for estimation of the intensity of this process, moreover, it can be used for the evaluation of oxidative stress severity (Halliwell and Whiteman, 2004). Lipids are most susceptible for peroxidative damage due to low energy necessary for the initiation of the process as well as the presence of unsaturated bonds (Balasinska, 2004). Our result of MDA is in agreement with many authors (Saleh *et al*., 2007; Andrei *et al*., 2009 and Yang *et al*., 2011b). Andrei *et al*. (2009) elucidated that dropping antioxidant concentrations is correlated with an increase in oxidation process at the level of lipids as demonstrated by the increasing concentration of MDA. Yang *et al*. (2011b) explained that higher levels of MDA in SCM cows demonstrated that the auto-oxidative activity in mastitic cows is higher than the healthy ones. Additionally, MDA is known to be mutagen and suspected carcinogic as it can react with DNA to generate mutations.

The SCM cows in our study recorded a significant increase in the levels of nitric oxide. NO production is considered as a primer defense system (Okamoto *et al*., 1997) as it has antimicrobial properties due to peroxynitrite, a reactive nitrogen metabolite, derived from oxidation of NO (Beckman *et al*., 1990), however, peroxynitrite can cause alteration in antioxidant balance in microorganism when produces in excess (Chaiyotwittayakun *et al*., 2002). During infections (such as mastitis or metritis) immune cells in the body recognize invading pathogens and become activated. Moreover, endotoxins released by bacteria activate immune cells. The host activated immune cells released inflammatory, mediators such as NO, (Bradford, 2011) which is previously called bioactive killing molecules (Shuster *et al*., 1997). Nitric oxide is a potent biological effector regulating blood vessel dilatation, serving as neuronal messenger, and plays a complex role in inflammatory response (Dawson and Dawson, 1995). The toxic effects of nitric oxide occur through the formation of peroxynitrite which is a powerful oxidant that causes chemical reaction in biological system including protein and DNA nitrosylation as well as lipid peroxidation (Murphy, 1999). So, in addition to MDA, NO is considered as another stressor which may be including in the resulted chromosomal aberrations through its direct effect on DNA causing damage.

On the other hand, the present investigation on SCM cows infected with *S.aureus* microorganism reported a significant decrease in the measured enzymatic antioxidants parameters, TAC, GSH-Px and CAT activities.

The measure of TAC considers the cumulative action of all the antioxidant present in plasma, thus providing an integrated parameter rather than the simple sum of measurable antioxidants. Also, as a

single measure, TAC provides relevant information that may effectively describe the dynamic equilibrium between pro-oxidants and antioxidant in the plasma compartment (Ghiselli *et al*., 2000). Mohamed (2007) indicated a reduction in both individual and total antioxidant status and increase in lipid peroxidation manifested by an increase in MDA in mastitic dairy camels. Our results are in agreement with the results recorded by Kleczknowski *et al*. (2005) and Ranjan *et al*. (2005) as in cows with SCM decreased potential of antioxidant protection in the blood was noticed. The last authors clarified that antioxidant status declines in inflammatory udder conditions, suggesting that incorporation of antioxidant may help in better management of mastitis in dairy cows.

Concerning the enzymatic antioxidants, GSH-Px and CAT, kale *et al*. (1999) explained that such enzymes may have important functions in alleviating the toxic effects of ROS. Plasma GSH-Px comprises intracellular antioxidant defense it catalyses the reduction of hydrogen and lipid peroxides protecting the cell membrane from oxidative damage caused by free radicals (Halliwell and Chirico, 1993). Also, As GSH-Px is a selenoenzyme; it has been observed that concentration of selenium and GSH-Px activity negatively correlated with the prevalence of intramammary infection (Erskine *et al*., 1987). In a recent study on SCM cows Karyak *et al*. (2011) recorded nonsignificant decrease in the activity of GSH-Px and revealed their results to low severity of inflammation. But our results contrast these results as in the present work, the affected dairy cows where undergo recurrent SCM and the recurrency of infection may be a reasonable cause for significant decrease in the activities of enzymatic antioxidant.

Regarding to the structural chromosomal aberration, it is the main type of aberrations observed; it may be occur under the effect of two main stressors. First of them is the bacterial toxins *(S.aureus* toxins and virulence factors), while the second is the oxidative stress (MDA and NO) which can damage all type of biomolecules including DNA and induce tissue damage (Zhao and Lacasse, 2008; Yang *et al*., 2011b). Here globally we accept the three theories of Assayed *et al*. (2010) who illustrated that the structural chromosomal aberrations resulted from: (1) direct DNA breakage, (2) replication on damaged DNA template or (3) inhibition of DNA synthesis.

CONCLUSION

The recurrency of subclinical mastitis in dairy cows under investigation may be attributed to more the one factor:

1- The presence of *S.aureus* pathogen which imbedded in the mammary gland producing its toxins and resisting the antimicrobial agents when growing in biofilms.

2- The cows undergo two main stressors first is the stress of lactation and the second is the stress of infection and inflammation which are a contributory factors for imbalance in oxidative status of cows.

3- The structural chromosomal aberrations that occur under the effect of the above stressors.

Finally, we can summarize our recommendation in three main points:

- Early prediction of the subclinical cases by developing a set of blood biomarkers that can reliably reflect tissue oxidation status in the individual animals.
- Following the program of eradication of *S.aureus* which can be summed up in sampling, culling, grouping and dry cow therapy.
- Increasing the performance of high yielding dairy cattle that optimized to a certain extent by supplementing diets with optimal levels of micronutrients with antioxidant capabilities.

REFERENCES

Abdel Hameed, K.G.; Sender, G. and Kossakowska, A.K. (2008): An association of Bola alleles DR B 3.2 * 16 and DRB 3.2*. 2*23 with occurrence of mastitis caused by different bacterial species in two herds

of diary cows. Animal science papers and reports Vol. 26, NO. 1. 37-48.

Aebi, H. (1983): Catalase. In methods Enzymology (Ed.H.U.Berg meyer). Pp. 276-286. Academic press, New York.

Ahlner, S. (2003): Prevalence of subcliincal mastitis in Uruguay. Aproject degree. Depart. Vet. Micro. Faculty of Vet. med., Uppsala Univ. 10-11.

Andrei, S.; Matei, S.; Fit, N.; Cernea, C.; Ciupe, S.; Bogdan, S. and Groza, I.S. (2011): Glutathione peroxidase activity and its relation with somatic cell count, number of colony forming units and protein content in subclinical mastitis cow milk. Romanian Biotechnological letters. Vol. 16, No. 3: 6209–6217.

Andrei, S.; Pintea, A.; Bunea, A.; Grozai, I.S.; Bogdan, S.; Ciupe, S.; Matei, S. and Crainic, D. (2009): Non-enzymatic antioxidant concentration and lipid peroxidation level in milk from cows with subclinical mastitis. Bulletin UASVM, Vet. Med. 66 (1): 196. 202.

Assayed, M.E.; Khalaf, A.A. and Salem, H.A. (2010): Protective effect of garlic extract and vitamine C against in vivo cypermethrin-induced cytogenetic damage in rate bone marrow. Mutation Res. 702: 1-17.

Balasinska, B. (2004): Evaluation of antioxidant Status in living organisms. Med. Weter. 60: 579-583.

Beckman, J.S.; Backmen, T.W. and CHEN, J. (1990): Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proceedings of the National Academy of Sciences of the USA, Vol. 87: 1620-1624.

Bhupal, G. (2007): Cytokine expression in milk somatic cell during mastitis in cattle and buffaloes. Thesis, Master of Vet. Sci., collage of Vet. Sci., and Anim. Husb. Anada Agric. Univ.

Bradford, B. (2011): Inflammation and transition cow disorders. Department of Anim. Sci. and Industry. Kanasa state Univ.

Bramely, A.J. and Dodd, F.H. (1984): Reviews of the progress of dairy science: Mastitis control progress and prospects. J. Dairy Res. 51, 481–512.

Chaiyotwittayakun, A.; Erskine, R.J. and Bartett, P.C. (2002): The effect of ascorbicacid and 1-histamine therapy on acute mammary inflammation in dairy cattle. J. Dairy Sci., 85: 60-67.

Dawsaon, T.M. and Dawson, V.L. (1995): Nitric oxide actions and pathological roles. Neuroscientist. 1: 7-18.

Erskine, R.J.; Eberhart, R.J. and Hutchimon, L.J. (1987): Herd management and mastitis prevalence in high and low cell count dairy herds. J. Am. Vet. Med. Ass., 190: 1411-1416.

Ghiselli, A.; Serafini, M. and Natella, F. (2000): Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free radical Biology and Medicine, 29: 1106-1114.

Halliwell, B. and Chirico, S. (1993): Lipid peroxidation: Its mechanism, measurement and significance. Am. J. clinic Nut. 57: 7155-725.

Halliwell, B. and Whiteman, M. (2004): Measuring reactive species and oxidative dmage in vivo and in cell culture: how should you do it and what do the result mean? Br.J. pharamacol. 142: 231- 255.

Kale, M.; Rathore, N.; John, S. and Bhatnagar, D.C. (1999): Lipids peroxidative damage on pyrethroid exposure and alteration in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. Toxicol. Lett. 105i 197-205.

Karyak, O.G.; Safi, S.; Rahimi froushani, A. and Bolourchi, M. (2011): Study of the relationship between oxidative stress and subclinical mastitis in diary cattle. Iranian J. Vet. Res. Shiraz University, Vol. 12, No.4, Ser. No. 37: 350- 353.

Kleczknowski, M.; Khucinski, W.; Shaktur, A. and Sikora, A.J. (2005): concentration of ascorbic acid in the blood of cows with sub clinical mastitis. Polish J.Vet. Sci., 8: 121-125.

Koracevic, D. and Koracevic, G. (2001): Determination of total antioxidant capacity in serum J. Clin. Path, 54: 356-361.

Macgregor, H.C. (1993): "An Introducation to Animal Cytogenetics". 1st Ed. Champ man and Ital. London Glasgow New York- Tokyo- Melbourne – Madras.

Melchior, M.B.; Fink–Gremmels, J. and Gaastra, W. (2006): Comparative assessment of the antimicrobial susceptibility of staphylococcus aureus isolated from bovine mastitis in biofilm versus planktonic culture. J.Vet. Med. - Berlin Vol. 53, No. 7: 326-332.

Mohamed, H.E. (2007): Antioxidant status and degree of oxidative stress in mastitis and healthy camel (Camelus dromedaries). Res. J. Anim. Sci. 1(3): 92-94.

Momtaz, H.; Rahimi, E. and Tajbakhsh, E. (2010): Detection of virulence factors in staphylococcus aureus isolated from clinical and subclinical bovine mastitis in Iran. African J. Biotech. 9 (25): 3753–3758.

Murphy, M.P. (1999): Nitric oxide and cell death. Biochem- Biophys. Acta. 1411: 401-414.

National Mastitis Council "NMC" (1999): Laboratory Handbook on Bovine Mastitis, Revised Ed., National Mastitis Council Inc., Madison.

Nicholas, F.W. (1996): "Introduction of veterinary Genetics". Oxford Univ. Press, Inc. New York.

Oh Kawa, H.; Ohish, W. and Yag, I.K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analchem 95: 351-358.

Okomota, T.; Akaike, T.; Nagano, T. (1997): Activation of human neutrophile procollagenase by nitrogen dioxide and peroxynitrite: a novel mechanism for procollagenase activation involving nitric oxide. Archives of Biochemistry and Biophysics, Vol. 342; 261-274.

Palige, D.E. and Valentine, W.N. (1967): J.lab. Clin. Med. 70: 158-169.

Rajaraman, V.; Nonnecke, B.J.; Franklin, S.T.; Hammell, D.C. and Horst, R.L. (1998): Effect of vitamin A and E on nitric oxide production by blood mononuclear leukocytes from neonatal calves fed milk replacer. J. Dairy SCi., 81: 3278- 3285.

Ram, S.V. and Arvid, B. (1995): "Human Chromosomes Principles and Techniques" 2nd Ed. International Edition Mc Graw Hill, Inc.

Ranjan, R.; Swarp, D.; Naresh, R. and Patra, R.C. (2005): Enhanced erythrocytic lipid peroxides and reduced plasma ascorbic acid and alteration in blood trace elements level in dairy cows with mastitis. Vet. Res. Commun., 29: 27-34.

Risco, C.A.; Donovan, G.A. and Hernandez, J. (1999): Clinical mastitis associated with abortion in dairy cattle. J. Dairy Sci. 82: 1684–1689.

Saleh, M.; Salam, A. and El-Mileegy, I.M.H. (2007): Oxidative antioxidative status during transition from late pregency to early lactation in native and cross bred cows in the Egyptian Oasis. Assiut Vet. Med. J. 53: 113.

Sharma, N.; Srivastava, A.K.; Basic, G.; Jeong, D.K. and Sharma, R.K. (2012a): Epidemiology. Bovine Mastitis Statish serial publishing house, Delhi, India: 231-312.

Sharma, N.; Rho, G.J.; Hong, Y.H.; Kang, T.Y.; Lee, H.K.; Hur, T.Y. and Jeong, D.K. (2012b): Bovin mastitis : An Asian Perspective. Asian Jourrnal of Animal and Veterinary Advances 7 (6): 454- 476.

Sharma, N.; Singh, N.K.; Singh, O.P.; Pandey, V. and Verma, P.K. (2011 b): Oxidative stress and antioxidant states during trasition period in dairy cow. Asiant- Aust. J. Anim. Sci. 24: 479- 484.

Sharma, N. and Matiti, S.K. (2010): Incidence, etiology and antibiogram of subclinical mastitis in cows in durg, chhattisgrah. Indian J. Vet. Res., 19: 45- 54.

Shuster, D.E.; Kehrli, M.E.; Rainard, P. and Paape, M.J. (1997): Complement fragment C5a and inflammatory cytokins in neutrophil recruitment during intrammary infection with E-coli. Infect. Immun. G5: 3286-3292.

Snedecor, G.W. and Cochran, W.C. (1982): Statistical methods". 8th Ed. Iowa State University press. Ames USA.

Sodeland, L.M.; Kent, M.P.; Olsen, H.G.; Opsal, M.A.; Svendsen, M.; Sehested, E.; Hayes, J. and liens, S. (2011): Quantitative trait loci for clinical mastitis on chromosome 2, 6, 14 and 20 in Norwegian Red cattle. Anim. Genet. Oct, 42 (5): 457- 465.

Sordillo, L.M. and Aitkin, S.L. (2009): Impact oxidative stress on the health and immune function of dairy cattle. Vet. Immunal. Inmunopathol. 128(1-3): 104-109.

Sordillo, L.M.; Contreras, G.A. and Aitken, S.L. (2009): Metabolic factors affecting the inflammatory res ponse of periparturient dairy cows. Anim. Health res. 10: 53-63.

Sordillo, L.M.; O`Boyle, N.; Gandy, J.C.; Corl, C.M. and Hamilton (2007): Shifts in thioredoxin reductase activity and oxidant status in mononuclear cell obtained from transition dairy cattle. J. Dairy Dci. 90: 1186-1192.

Sordillo, L.M. (2005): factors affecting mammary gland immunity and mastitis susceptibility. Livest. Prod. Sci. 98: 89-99.

Spears, J.W. and Weiss, W.P. (2008): Role of antioxidants and trace elements in health and immunity of transition dairy cows. The Vet. J. 176 (1): 70-76.

Toply and Wilson (1998): Microbiology and microbial infections. Ninth Edition. Leslie Collier Aibert Balow. Max Sussman. Co-published in the USA by Oxford Univ. Press Inc.

Yang, F.L.; Li, X.S.; He, B.X.; Du, Y.L.; Li, G.11.; Yang, B.B. and Qin–Hua, H. (2011a): Bovine mastitis in subtropical dairy farms. J. Anim. Vet. Adv., 10: 68-72.

Yang, F.L.; Li, X.S.; He, B.X.; Yang, X.L.; Li, G.H.; Liu, P.; Huang, Q.H.; Pan, X.M. and Li, J. (2011b): Malondialdehyde level and some enzymatic activities in subclinical mastitis milk. Afriacan J. Biotech. Vol.10 (28): 5534–538.

Yong, Z.; Fang, J.X.; Mei, Y.; Narisu, S. and Binzhong, L. (2009): Isolation and Identification of pathogens from mastitis cow and drug sensitivity test. China Anim. HUsb. Vet. Med., 36: 136–140.

Zhao, X. and Lacasse, P. (2008): Mammary tissue damage during bovine mastitis: causes and control. J. Anim. Sci. 86 (1): 57-65.