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COMPARATIVE STUDY OF CONCENTRATION LEVEL OF OXIDATIVE STRESS BIO MARKERS IN BLOOD FOLLICULAR FLUID AND MUCOUS OF CERVIX IN CATTLE SUSPECTED WITH OVARIAN FOLLICULAR CYST

Eftkar Mahdy Al-Najar*, Talib Ahmed Hamid and Makki Khalaf Hussein
Technical College AL-musaib, AL-Furat AL-Awsat Technical University, Iraq
*Corresponding author Email: iftkaralnajar @atu.edu.iq

Abstract

One hundred samples (blood, follicular fluid, cervical mucus) was collected from cows suspected with cystic ovary at time of slaughter for a period from sept. 2017 to sept. 2019 in Aldora slaughtering center / Baghdad. All samples centrifugation 3000 rpm / min. then refrigerator at -20 ºC until analysis with biochemical test, MDA measured by Yagi method Glutathione measured by enzymatic recycling method. Catalase assessed according to method of Chohen *et al*. (1970) Vitamin C and vitamin E measured by idometric and automated method respectively. The results of this study showed significant decreasing P <0.001, P <0.05 of antioxidant in samples of cystic ovary cows compared with ovulated. While the concentration of MDA (stress biomarker) was increase significantly P<0.001 in blood and cervical secretion except the follicular fluid showed significant decreasing P<0.001 in cystic ovary cows compared with ovulated. The results appeared that cystic ovary is a stress state illustrated by disruption of oxidant / antioxidant balance compared with ovulated cows the concentration of antioxidant and oxidative marker was alter according to their importance in the process of ovulation events.
Keywords: Oxidative stress, bio markers, blood follicular, ovarian follicular cyst.

Introduction

Ovarian follicular cyst is a common reproductive disorder in cattle (Talukder *et al.* 2014). The typical incidence of this disorder in cows is about 3% (Silvia *et al.*, 2002). It's characterized by increasing growth of dominant follicle to more than 2.5 cm in size with a falture to ovulate to become a persistent follicular structure in the absence of a function of corpus luteum (Mwaanga and Janowski, 2000). Reactive oxygen species (Ros) and antioxidants remain in balance to maintain the cellular homeostasis but when this balance is altered as a result of antioxidant depletion or increase in Ros production OS occurs (Agarwal *et al*., 2005). The recent study demonstrate that OS can affect a variety of physiological functions in the female reproductive system, Like follicular fluid environment, folliculogenesis and steroidogenesis and generally high levels of Ros can disrupt several reproductive events that may result in adverse pregnancy outcomes (Al-Gnbory *et al*., 2010). Os has a role in pathogenesis of follicular cystic ovarian disease (Rizzo *et al.,* 2009) and cervical factor infertility (Speroff *et al*., 1999). Therefore the objective of this study was to examine the plasma, follicular and mucous concentration of os bio markers in cows diagnosed with an ovarian cyts (Celli *et al*., 2912).

Materials and Methods

Collection of blood samples. The samples of blood was collected from jugular vein of pre slaughter cows suspected by C.O using 5 ml disposable syringes and with allow to clot and storage at -20 ºC (Bioshop *et al*., 2000). Collection of cervical secretions about 0.5 ml of cervical secretions was liking by syringe from cervix of cows using vaginal speculum, labeled and storage at -20 ºC for biochemical test. The mucous most be liquefied by mucolytic agent of N-acetyl L- cysteine of concentration 0.2 mg/ml which prepared by weight 0.2 mg of N- acetyl L- cysteine and complete to one mililiter with Foulkes *et al*. (1981). Collection of follicular fluid :- ovaries collected from cows at the time of slaughter and divided into two groups according to the size of follicle , the first group large than 2.5 cm classify as cysted follicle (non-ovulated), and smaller than 2.5 cm as (ovulated) follicle, the follicular fluid aspirated from the two groups of follicles by using disposable tube and store in -20 ºC for next analysis (Arshad *et al.*, 2005).

Biochemical test (malondialdehyde)

1. (MDA) test: was measured in blood follicular fluid and cervical mucus according to yagi method 1998.
2. Glutathion GSH: measured by an enzymatic recycling method according to information of (sigma Aldrich pty ltd, castle, hill, NSW, Australia) (Baker *et al*., 1990).
3. Catalase: Measurement of Catalase activity in blood follicular fluid and cervical secretion according to manor of (Chohen *et al*., 1970).
4. Vitamin C: measured by iodometric titration according to (Nwezo *et al*., 2015).
5. Vitamin E: measured by automated methods according to (Yuji *et al*., 2014).

Results

The level of MDA is serum and cervical secretion showed significant increase in cows compared with ovulated cows while its level of concentration in follicular fluid shows significant decrease in CO cows compared with ovulated as shown in table (1).

Table 1 : The MDA levels in sera, follicular fluid and cervical secretion in ovulated and cystic ovary cow.

Sample	Ovulated Mean ± SD MM	Cystic ovary cows	P value
Serum MDA	1.923± 0.417	3.504 ± 1.443	P < 0.001
Follicular MDA	1.733 ± 0.511	0.933 ± 0.411	P < 0.001
Cervical secretion MDA	3.331 ± 1.022	6.971 ± 3.011	P < 0.001

The glutathione levels concentration in serum, follicular fluid and cervical secretions in CO demonstrate a significant decrease (P<0.05, P<0.05, P<0.001) receptivity compared to ovulated cows as shown in table (2).

Table 2 : Glutathione level in serum , follicular fluid and cervical secretion of PCO cow and ovulated.

Sample	Ovulated cow Mean ± SD MM	Cystic ovary cows Mean ± SD MM	P value
Serum glutathione	28.348± 8.457	12.798 ± 8.460	P < 0.05
Follicular Fluid glutathione	30.330 ± 12.464	12.597 ± 6.324	P < 0.05
Cervical secretion glutathione	67.326 ± 18.216	48.601 ± 15.353	P < 0.001

The catalase level concentrations in serum, follicular fluid and cervical secretions of B.O.C showed a significant decrease (P < 0.05) when compared with ovulated cows as shown in table (3).

Table 3 : Catalase level concentration in serum, follicular fluid and cervical secretions in BOC and ovulated.

Sample	Ovulated cow Mean ± SD K/ml	Cystic ovary cows Mean ± SD K/ML	P value
Serum catalase	0.579± 0.343	0.033 ± 0.275	P < 0.05
Follicular Fluid catalase	0.497 ± 0.342	0.303 ± 0.286	P < 0.05
Cervical secretion catalase	0.508 ± 0.301	0.332 ± 0.303	P < 0.001

Vitamin C level in serum, follicular fluid and cervical mucus of cystic ovary cow showed significant decrease P < 0.05 compared with ovulated cow as shown in table (4).

Table 4 : Vitamin C level concentration in serum, follicular fluid and cervical mucus in cystic ovary cows and ovulated.

Sample	Ovulated cow Mean ± SD mg/ l	Cystic ovary cows Mean ± SD mg/l	P value
Serum v. c	13.377± 3.545	9.933 ± 1.643	P < 0.05
Follicular Fluid v.c	10.879 ± 3.334	6.281 ± 1.334	P < 0.05
Cervical secretion v. c	20.145 ± 4.042	12.233 ± 3.485	P < 0.05

Vitamin E level in serum, follicular fluid and cervical mucus of cystic ovary cow show a significant decrease P<0.05, P<0.001, P<0.001 respectively compared with ovulated cows as shown in table (5).

Sample	Ovulated cow Mean ± SD mg/ l	Cystic ovary cows Mean ± SD mg/l	P value
Serum V. E	10.334± 3.536	5.273 ± 1.445	P < 0.05
Follicular Fluid V. E	6.224 ± 0.651	4.332 ± 0.631	P < 0.001
Cervical secretion V. E	7.033 ± 0.764	4.633 ± 1.434	P < 0.001

Discussion

The results of this study demonstrated that cystic ovary in cow is oxidative stress state because of disruption between antioxidant/oxidant balance (Rizzo *et al*., 2012). This disruption is associated with decrease of enzymatic and non enzymatic antioxidant (Glutathione, catalase, vitamin c and vitamin E), In each blood, follicular fluid and cervical secretion while there was increase in MDA (The stress biomarker) as a metabolite product of lipid peroxidation (Hatice *et al*., 2017). IN serum and vaginal secretion except the follicular fluid that means there was decreasing of ROS to under physiological level required to ovulation. So it will be insufficient to breakdown the follicle wall tend to transformation of preovulatory follicle in to follicular cyst (Shama and Agarwal, 2004). The disruption between oxidant/antioxidant balance prevented the necessary physiological response involved in inflammatory reaction which trigger the follicle rupture and hens ovulation, Glutathione is the main indicator of free radical scavenging ability of all fluid so its decrease in cystic ovary cow results from depletion into protect the body against Ros harmful effects, as well as catalase antioxidant enzyme which prevented a reduction in oocyte penetration of bull sperm in the presence of reactive oxygen species (Gabia *et al*., 2004). It appear that its deficiency in cystic ovary cow may prevent or alter ovulation events of dominant follicle resulting in ovarian cyst (Rezzo *et al*., 2009).

Vitamin C associated with vitamin E and glutathione in playing main role in biosynthesis of steroid and peptide hormone indirectly ascorbic acid plays important regulatory roles through the enteric body due to its involvement in synthesis of hormones, hormone releasing factor and neurotransmitter (Seyfullah *et al*., 2008).

This is to be clear them decreasing in all sample of cystic ovary cow compared to ovulated cow lead to hormonal disturbance hens cystic ovary (Shama and Agarwal, 2004).

Reference

Agarwal, A.; Gupta, S. and Sharma, R. (2005). Role of oxidative stress in female reproduction reprod boil. Endocrinal. 3: 21.

Al-Gubory, K.H.; Fowler, P.A. and Garrel, C. (2010). The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. Int. J. Biochem. cell. Biol. 42: 1634- 1650.

Arshad, H.M.; Ahmed, Z.R.; Samad, H.A. and Akhtar, N. (2005). Study of some biochemical concentrate of ovarian follicular fluid and peripheral blood in buffaloes, Pakistan, Vet., 25 (4).

Baker, M.A.; Cerniglia, G.J. and Zaman, A. (1990). Microliter plate assay for the measurement of glutathione and glutathione disulfide in large number of biological samples. Anal. 190: 360–365.

Chohen, G.D.D. and Judith, M. (1970). Measurement of catalase activity in tissue extract analytical biochemistry, 34: 30–38.

Bioshap, M.P.; Jeffery, S.; Kargel, H.H.; Kiffer, D.M. (2000). Annale of glaciology. 31: 169–170.

Celi, P.; Merlo, M.; Borbato, O. and Gabai, G. (2012). Relationship between oxidative stress and the success of artificial insemination in dairy cows in apassture based system Vet J., 193: 484-502.

Foulker, J.A. *et al* (1981). Res. Vet. Sci. bovine cervical mucus peroxide concentration at oestrus.

Gabia, G.; Testoni, S.; Piccinini, M.I. and Estradiol (2004). Oxidative stress in primiparous cows in relation to dilatory starch and progress of lactation. Anim. Sci., 79: 99-108.

Hatice, E.; Murad, O. and Sukru, K. (2017). MDA and GSLI–PX activity in transition diary cows under seasonal variation and the ir-relationship with reproductive performance. Journal of Vet. Res., 61(4): 497–502.

Mwaanga, E. and Janowski, T. (2000). Anoatrus in dairy cows : causes prevalence and clinical from reprod domest Anim., 35: 193- 200.

Nweze, M.; Abdulgamiyu, G. and Erhabor, O.G. (2015). Comparative analysis of vitamin C in fresh fruits juice of *Malus domestica* , *Citrus sinensis*, *Ananas comosus* and *Citrullus lanatus* by iodometeric titration. International journal of science. Environment and technology, 4: 17- 22.

Rizzo, A.; Minoia, G.; Trisolini, C.; Mutinanti, M.; Spedicate, M.; Jirillo, F. and Sciorsic, R.L. (2000). Reactive oxygen species (ros) involvement in bovine follicular cysts etiopathogenesis immunopharmacol Immunotoxicol, 3: 631- 635.

Rizzo, A.; Romscino, M.T.; Binette, F. and Sciorsci (2012). Roles of reactive oxygen species in female reproduction. Rerod. Domestic animal. 47: 344–352.

Seyfullah, H.; Huseyin, E.B.; Serpek and Tevfic, T. (2008). The relationship among vitamin C, B, Carotene, vitamin A progesterone and estradiol 17-B concentration in plasma and cyst fluid of holestein cow with ovarian cyst, Rep in domestic animal, 43(5): 573–7.

Shama, R.K. and Agarwall, A. (2004). Role of reaction oxygen species gynecologic disear, Review article rerod, Med, Biol. 3: 177-199.

Silvia, W.S.; Halter, T.B.; Nugenl, A.M.; Laranja, D.A. and Fonseca, L.F. (2002). Ovarian follicular cysts in dairy cow abnormality in folliculogenesis domestic animal endocrinal. 23: 167–177.

Talukder, L.; Ingenheff, K.L. and Kerrish, P. (2014). Cell plasma oxidative stress biomarkers and progesterone profiles in a dairy cow diagnosed with an ovarian follicular cyst. Veterinary quarterly, 34(2): 113-117.

Yagi, K. (1998). Sample procedure for specific assay of lipid hydroperoxides in semen or plasma method in molecular biology, 108: 107-110.

Yuji, H.; Hiroshi, Y. and Norio, T. Antomated measurement method for determination of vitamin E in plasma lipoprotein classes, 421-429.