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COMPARATIVE ANALYSIS OF ANTIOXIDANT ACTIVITY AND REPRODUCTIVE HORMONES IN POSTPARTUM ANESTRUS COWS DIAGNOSED BY ULTRASOUND IN BASRAH PROVINCE

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Abstract

This study was conducted in Basrah province from September 2022 till December 2022 to evaluate the ratio of enzymatic antioxidants; catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-px), glutathione (GSH), and the concentration of malondialdehyde level (MDA) in the serum of 10 cows that suffered from postpartum anestrus compared five cyclic cows as a control group. In addition, a hormonal evaluation (Estrogen E2, progesterone P4, and cortisol) in postpartum anestrus cows compared regular control group. Postpartum anestrus and cyclic cows are diagnosed ultrasonographically by examining the genital tract per rectum using ultrasound. The results revealed a significant decrease ($P<0.05$) in all antioxidant enzymes (SOD, CAT, GSH-px) in postpartum anestrus cows in compared with to regular cyclic cows. The results of GSH analysis revealed that non-significant elevation occurred in postpartum anestrus cows compared to normal cyclic cows, and a significant increase ($P<0.05$) in the MDA level occurred in anestrus cows compared control group. The hormonal analysis showed a significant decrease ($P<0.05$) in both estrogen and progesterone with a significant elevation ($P<0.05$) for cortisol in anestrus cows compared to cyclic cows. The study concluded the postpartum anestrus cows were under oxidative stress, which plays an essential role in anestrus, especially after parturition..

Keywords: *Enzymatic Antioxidant, Reproductive Hormones, Postpartum Anestrus.*

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Introduction

Postpartum anestrus in cows is regarded as one of the major reproductive diseases that affect the estrus cycle, followed by the economic waste in dairy cows (1). It is considered a usual case associated with some physiological conditions, such as early postpartum and milking periods. It is classified as an abnormal pathological problem when it exceeds the normal open days (2).

During pregnancy; progesterone suppresses the hypothalamus-pituitary axis and decreases Gonadotropin Releasing Hormone (GnRH) production (3). Increased levels of GnRH after delivery from the hypothalamus and FSH-LH from the anterior pituitary increase follicular development and continued cyclicity (4). However, the recovery of the hypothalamic-pituitary axis and initiation of ovarian activity may be delayed and prolong the period of postpartum anestrus in cattle (4). Many causes delay the period of restoring ovarian cyclicity after delivery, like aging, stress, climate changes, and nutritional condition (5).

Many studies indicated the relationship between stress factors and animal reproductive ability (6, 7, and 8). Stress factors like climate changes, nutritional deficiencies, milk production, healthy condition, and prevalent diseases are essential in producing reactive oxygen species (ROS) and reactive nitrogen species (RNS) (9, and 10). ROS and RNS include hydroxyl radicals, superoxide ions, hydrogen peroxide, and nitric oxide radicals, which affect the fertility of the animals (11). Generally, ROS and RNS affect folliculogenesis and steroidogenesis, which are essential for the reappearance of estrus within normal open days after delivery (12, and 13). Many studies revealed the relationship between antioxidant enzymes and the incidence of retained placenta in cows (14) and buffaloes (15), polycystic ovaries (16), and infertility in male (17) and female (18).

Antioxidant defense systems (enzymatic and non-enzymatic) resist the adverse effect of free radicals by neutralizing these ROS and minimizing the free radical impact on viable cells (19). The enzymatic antioxidants include CAT, SOD, and GSH-px. In contrast, the non-enzymatic antioxidant contains GSH, vitamin C, vitamin E, β- carotene, and different elements (15-19). Therefore, the present study evaluated the differences in the activity of enzymatic antioxidants and their relationship with some reproductive hormones in cows suffering from postpartum anestrus compared to normal cyclic cows.

Materials and Methods

1. Animals study

The study included 15 cows; 10 of them suffered from postpartum anestrus (didn't exhibit any signs of estrus even after 100 days of birth), and five normal cyclic cows (in the estrus phase) were used as a control. Ultrasonography diagnosed postpartum anestrus cases from normal cyclic cows and the owners' clinical signs. The ultrasonography used CHISONR (ECO1, China) 5.0 MHz linear rectal probe to evaluate the reproductive system, especially ovarian activity and uterine status (8).

2. Antioxidant and oxidant parameters

The evaluation of CAT, SOD, and (GSH_Px) activity and the level of GSH) and MDA (as well as hormonal analysis for estradiol, progesterone, and cortisol) was done by collecting 10 ml of blood via the jugular vein of experimental animals. Serum was separated by centrifuge at 3000 rpm for 15 minutes and kept at – 20℃ until analysis. The activity of CAT enzyme (units/ml) in serum was determined according to the method of (20); modified by (21) by using a special kit (Sza kits, Germany) which depends on the amount of enzymes that catalysis the reaction of one µmol of hydrogen peroxide substrate per minute at 240 nm after 30 seconds. Total SOD and GSH-px activity in serum were measured based on (22) by using a special kit (Sza kits, Germany). These methods depend on the ability of SOD to inhibit epinephrine oxidation at 480 nm immediately and after 5 minutes at 37 C. while the activity of GSH-px depends on the amount of enzymes that catalysis the reaction of one µmol of Allman's reagent substrate at 412 nm and 37°C. The concentration of GSH (Mmole/L) was evaluated according to (23); modified by (24) by using a special kit (Sza kits, Germany) that used Allman's reagent at 37 C, and the absorbance was recorded at 412 nm. The concentration of MDA (Mmole/L) was done based on (25); modified by (26) by using (Sza kits, Germany), and the reaction with thiobarbituric acid was measured at 532 nm at room temperature. Enzyme assays did all previous analyses by spectrophotometer, apple, Japan.

3. Hormonal analysis

Progesterone (P4), estradiol (E2), and cortisol were estimated by Enzyme-Linked Immune Sorbent Assay (ELISA) technique by using a specific bovine kit (Yingxin Laboratory, China) according to the instruction of the manufacturer.

4. Statistical analysis

Statistical analyses were performed using student T-test through SPSS computer package version 25. The differences are considered to be significant at ($p<0.05$) (SPSS, Version 20, IBM, USA).

Results

The ultrasonography results revealed its efficiency for detecting postpartum anestrus cases compared to normal cyclic cows. Postpartum anestrus cows showed ovarian inactivity (inactive ovaries or anovulatory follicles), which appeared as a gray iso-echoic mass with echoic borders in the transverse section compared to normal cyclic cows, which revealed hypo-echoic mature or secondary follicles on the ovary as shown in (figure 1). Uterine horns examination with ultrasound for postpartum anestrus cows revealed a hyperechoic structure than other tissue in the transverse section compared to cyclic cows, which showed gray transparent echoic uterine layers with lumen as shown in (figure 2).

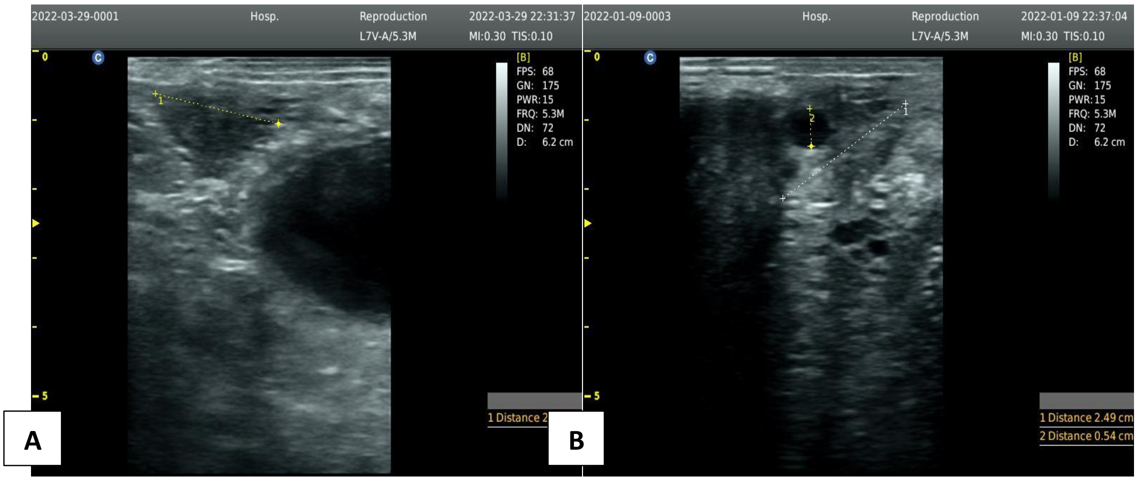

Figure 1. A, An ultrasound image of the ovary in postpartum anestrus cows shows gray iso echoic with a high echoic border and absence of follicles by using a 5 MHz trans-rectal transducer. B, Ultrasound images in normal cyclic cows shows hypo-echoic mass with mature follicles using a 5 MHz trans-rectal transducer.

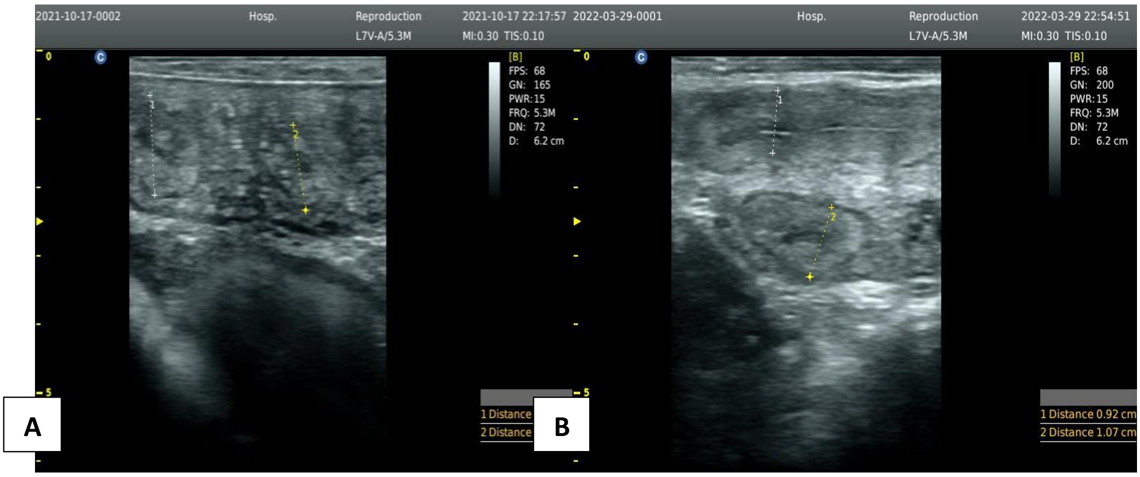

Figure 2. A, An ultrasound image of uterine horns is hyper echoic without a clear lumen due to ovarian inactivity in postpartum anestrus using a 5 MHz trans-rectal transducer. B, An ultrasound image of uterine horns shows gray transparent echoic uterine layers due to ovarian activity in cyclic cows by using 5 MHz trans-rectal transducer.

The results of the evaluation of antioxidant enzymes in postpartum anestrus cows revealed these animals were in a stress factor which showed a significant decrease (P<0.05) in CAT and SOD (figure 3) and GSH_Px (figure 4) compared to normal cyclic cows which showed sufficient levels of previous antioxidant enzymes.

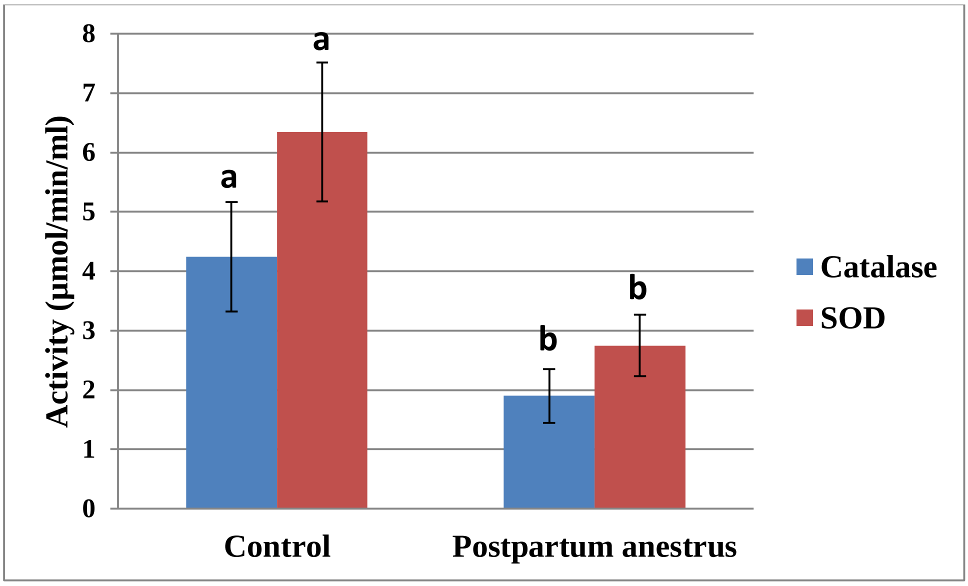

Figure 3. Catalase and SOD activity in postpartum anestrus cows compared normal cyclic cows. Different litters indicate significant difference at (p<0.05).

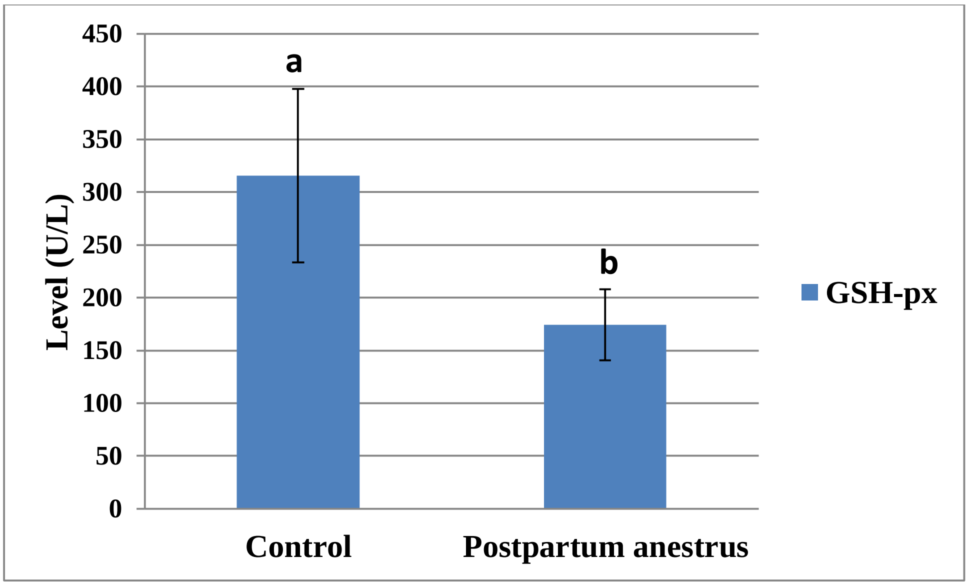

Figure 4. GSH-px activity in postpartum anestrus cows compared normal cyclic cows. Different litters indicate significant difference at (p<0.05).

The present study confirms the results of antioxidant enzymes through the evaluation of the concentration of GSH. The concentration of GSH in postpartum anestrus cows was (246.23±64.7), which is significantly (P<0.05) lower than normal cyclic cows (567.51±114.72), as shown in (figure 5).

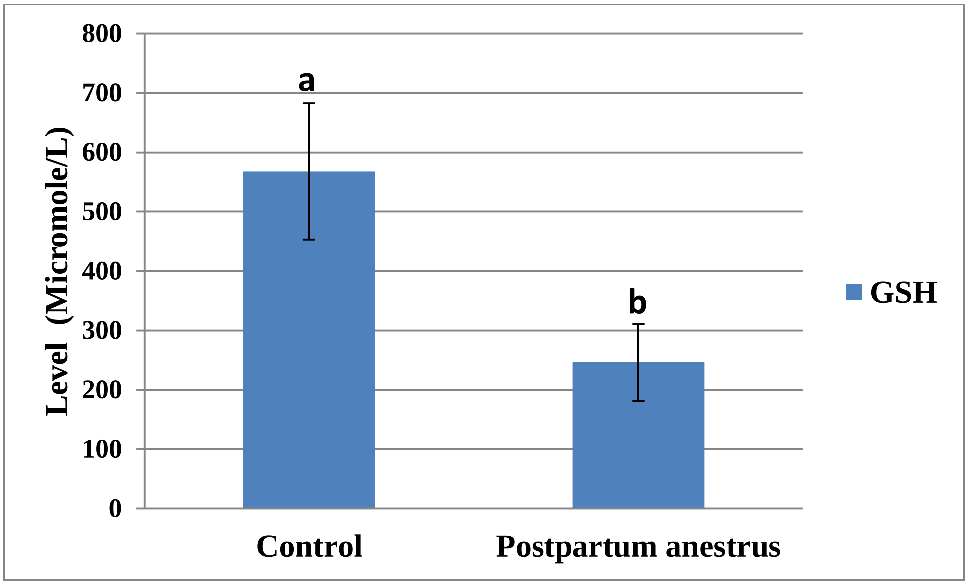

Figure 5. GSH level in postpartum anestrus cows compared normal cyclic cows. Different litters indicate significant difference at ($p<0.05$).

In the current study, the marker of oxidative stress was evaluated by estimating the level of MDA (the product of polyunsaturated fatty acid peroxidation in cells). The results revealed that the animals were under severe stress factors through the significant increase ($P<0.05$) in the level of MDA (90.44± 12.5) in postpartum anestrus cows compared to normal cyclic cows (61.08±11.88) as shown in (figure 6).

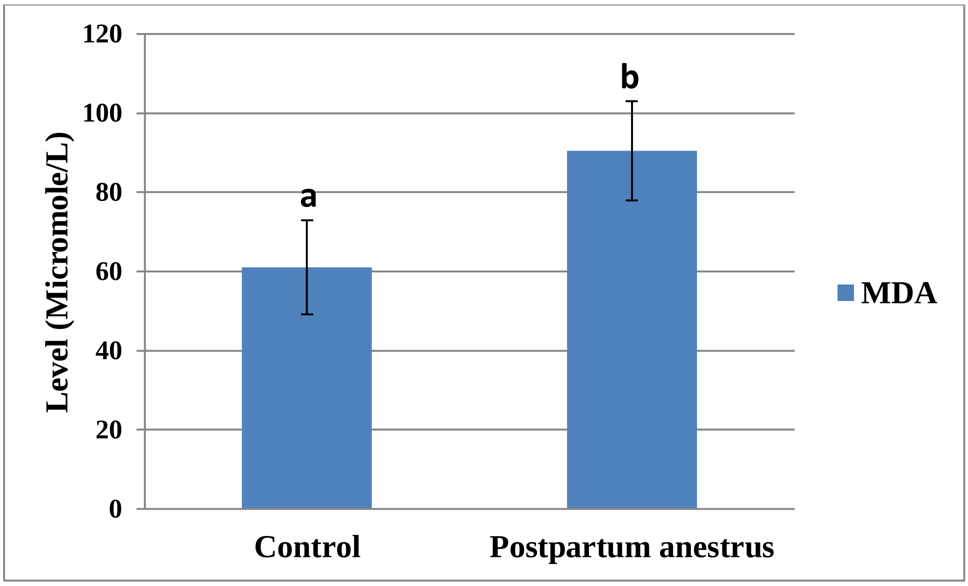

Figure 6. MDA level in postpartum anestrus cows compared normal cyclic cows. Different litters indicate significant difference at ($p<0.05$).

The results of the hormonal analysis confirmed that the cases of postpartum anestrus diagnosed by ultrasound device had a significant decrease ($p<0.05$) in the levels of estrogen compared to normal cyclic cows with the non-significant difference in the concentration of progesterone as in (table 1), and a slight rise in the concentration of cortisone in postpartum anestrus cows compared normal cyclic cows as shown in (table 1).

Table 1. concentration of hormones (estradiol, progesterone, and cortisol) in postpartum anestrus cows compared normal cyclic cows

Groups/Cows	Estradiol (pg/ml)	Progesterone (ng/ml)	Cortisol (pg/ml)
Postpartum anestrus	13.76±2.90 **a**	0.78±0.22 **a**	73.05±12.77 **a**
Normal cyclic	67.53±11.82 **b**	1.13±0.34 **a**	51.33±9.71 **a**

Data are presented as number (mean ± SEM). [ab] different letters within each column indicate significant difference ($p < 0.05$).

Discussion

Postpartum anestrus indicates that the cows have not returned to estrus despite exceeding the standard open days period, estimated at 60-80 days post-delivery (2 and 27). In a recent study, postpartum anestrus in cows formed more than 50% of the reasons for anestrus suffering from the luteal cyst, persistent corpus luteum, cystic corpus luteum, and uterine infection (8). The present study indicated the efficiency of ultrasound in diagnosing the presence and the causes of postpartum anestrus in cows, which agreed with many studies (8 and 28).

The current study evaluated the antioxidant enzymes CAT, SOD, and GSH_Px in all cases of postpartum anestrus cows compared to regular cyclic cows. The results revealed significant decreases in these enzymes compared to normal cyclic cows. Antioxidant enzymes induce the catalytic transformation of ROS and their by-products into other nontoxic chemicals and produce a defense mechanism against oxidative stress that may lead to cell damage (15 and 19). Deficiencies in the antioxidant enzymes are related to many diseases in humans and animals (14-18). The lack of enzymatic and non-enzymatic antioxidants allows reactive oxygen species (ROS) to react with cells and organs by oxidation or inducing chemical transformation, followed by many disorders (8, 14-18). Our results revealed the postpartum anestrus cows were in oxidative stress, which may have occurred due to heat stress, abnormal puerperium, milking, and insufficient diet intake (6, 7, and 9).

In our study, the low serum CAT, SOD, and GSH_Px levels in postpartum anestrus cows agreed with (29), who reported similar evidence in anestrus cows. A similar observation was seen in buffaloes (30) and ewes (31); they found a significant decrease in enzymatic antioxidants in anestrus animals compared to normal cyclic ones. (14) Their study revealed a significant decline in the level of enzymatic antioxidants in retained placenta compared to non-retained placenta animals. This fact indicates that postpartum anestrus cows were in oxidative stress, and the decrease in the levels of these enzymes may be due to the ongoing requirements for prevention of oxidative stress that is occurred after parturition to prevent free radicals formation and minimize their adverse effect (32).

In addition to enzymatic antioxidants, our results revealed a significant decrease in the level of GSH, which came in agreement with studies conducted on cows (19 and 29), buffaloes

(30) and ewes (31). GSH is the primary antioxidant produced by the cells and neutralizes ROS through its associated enzymes, like glutathione peroxidase, reductase, and transferase (11 anf 12). The reduction in the GSH level in postpartum anestrus cows confirms the animals studied were under oxidative stress, which interfere with normal endocrine function after parturition followed by postpartum anestrus (11).

The incidence of oxidative stress in animals study was documented after measuring the MDA level, which showed a significant increase in postpartum anestrus compared to normal cyclic cows. (30) Found a sharp elevation in MDA in anestrus water buffalo, and a higher level for MDA occurred in anestrus and repeat breeding in Cholistani cattle (29). MDA is an index of lipid peroxidation and a marker of oxidative stress in tissue systems (33).

The present study revealed a significant decrease in serum estradiol levels (but not for progesterone) in postpartum anestrus cows compared to normal cyclic cows, which is corroborated with many researches in cows (2,4 and 5), buffalos (35), and sheep (36). In anestrus cows, the disappearance of the follicular phase and follicular development plays an essential role in decreasing estradiol which is necessary for controlling the estrous cycle and sexual behavior of animals (2, 5). Bovine postpartum anestrus can be an issue due to poor nutrition, unfavorable weather, lack of energy, high milk production, management stress, parasites, and infections (5 and 37). (38) Investigated the role of thyroid gland hormones as regulators of ovarian function, especially after parturition. Our results showed a tight relationship between these causes and enzymatic antioxidant activity in postpartum anestrus cows. An apparent reduction in the levels of antioxidant enzymes occurred in postpartum anestrus cows compared to normal cyclic cows, which coincided with a decrease in the level of estrogen and, at the same time, increasing in the level of cortisol, which indicates the stress factor that animals suffered from them. Many studies in cows (39) and buffaloes (40) revealed the role of cortisol throughout stress, which tends to elevate as a response to resisting the stress factor and minimizing its effect on the body.

References

[1] Ambrose, D. J. (2021). Postpartum anestrus and its management in dairy cattle. Bovine Reproduction, 408-430.

[2] 2- Madhuri, G., Rajashri, M., & Kesharwani, S. (2017). Post-partum anoestrus in dairy cows: a review. International Journal of Science, Environment and Technology, 6(2), 1447-1452.

[3] 3- Crowe, M. A., Diskin, M. G., & Williams, E. J. (2014). Parturition to resumption of ovarian cyclicity: comparative aspects of beef and dairy cows. Animal, 8(s1), 40-53.

[4] 4- Cheong, S. H., Filho, O. G. S., Absalón-Medina, V. A., Pelton, S. H., Butler, W. R., & Gilbert, R. O. (2016). Metabolic and endocrine differences between dairy cows that do or do not ovulate first postpartum dominant follicles. Biology of Reproduction, 94(1), 18-1.

[5] 5- Kumar, P., Rajanna, R., & Sunitha, R. (2020). Anoestrus in bovines: A review article. The Pharma Innovation Journal, 9(9), 458-460.

[6] 6- Khodaei-Motlagh, M., Shahneh, A. Z., Masoumi, R., & Derensis, F. (2011). Alterations in reproductive hormones during heat stress in dairy cattle. African Journal of Biotechnology, 10(29), 5552-5558.

[7] 7- Dobson, H., Routly, J. E., & Smith, R. F. (2020). Understanding the trade-off between the environment and fertility in cows and ewes. Animal Reproduction, 17.

[8] 8- Hameed, W. S., & Alsalim, H. A. (2022). Ultrasonographical and hormonal comparative between true and postpartum anestrus of cows in south of Iraq. International Journal of Health Sciences, 6(S6), 7909–7925.

[9] 9- BV, S. K., Ajeet, K., & Meena, K. (2011). Effect of heat stress in tropical livestock and different strategies for its amelioration. Journal of stress physiology & biochemistry, 7(1), 45-54.

[10] 10- Chauhan, S. S., Rashamol, V. P., Bagath, M., Sejian, V., & Dunshea, F. R. (2021). Impacts of heat stress on immune responses and oxidative stress in farm animals and nutritional strategies for amelioration. International journal of biometeorology, 65, 1231-1244.

[11] 11- Ghosh, M., Gupta, M., Kumar, R., Kumar, S., Balhara, A. K., & Singh, I. (2015). Relation between antioxidant status and postpartum anestrous condition in Murrah buffalo. Veterinary world, 8(10), 1163.

[12] 12- Cruz, M. H. C., Leal, C. L. V., Cruz, J. F. D., Tan, D. X., & Reiter, R. J. (2014). Essential actions of melatonin in protecting the ovary from oxidative damage. Theriogenology, 82(7), 925-932.

[13] 13- Khan, A., Dou, J., Wang, Y., Jiang, X., Khan, M. Z., Luo, H., ... & Zhu, H. (2020). Evaluation of heat stress effects on cellular and transcriptional adaptation of bovine granulosa cells. Journal of animal science and biotechnology, 11(1), 1-20.

[14] 14- Khudhair, N. A., Abbas, H. R., & Alsalim, H. A. (2021). Relationship between enzymatic antioxidant activities and reproductive hormones in the cows with retained placenta in Basrah province, Iraq. Archives of Razi Institute, 76(5), 1537-1543.

[15] 15- Atallah, S., & Moustafa, S. (2006). Relation between oxidative stress and retained placenta in buffaloes. Assiut Veterinary Medical Journal, 52(110), 298-311.

[16] 16- Papalou, O., M Victor, V., & Diamanti-Kandarakis, E. (2016). Oxidative stress in polycystic ovary syndrome. Current pharmaceutical design, 22(18), 2709-2722.

[17] 17- Tvrdá, E., Kňažická, Z., Bárdos, L., Massányi, P., & Lukáč, N. (2011). Impact of oxidative stress on male fertility—A review. Acta veterinaria hungarica, 59(4), 465-484.

[18] 18- Bhardwaj, J. K., Panchal, H., & Saraf, P. (2021). Ameliorating effects of natural antioxidant compounds on female infertility: a review. Reproductive sciences, 28, 1227-1256.

[19] 19- Kankofer, M. (2001). Antioxidative defence mechanisms against reactive oxygen species in bovine retained and not-retained placenta: activity of glutathione peroxidase, glutathione transferase, catalase and superoxide dismutase. Placenta, 22(5), 466-472.

[20] 20- Beers, R. F., & Sizer, I. W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol chem, 195(1), 133-140.

[21] 21- Aebi, H. (1984). Catalase in vitro. In Methods in enzymology. 105, 121-126, Academic press.

[22] 22- Flohé L, Günzler W. (1984). Assays of glutathione peroxidase. Meth Enzymol. 105:114-20.

[23] 23- Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry, 25, 192-205.

[24] 24- Tietz NW, Pruden EL, Siggaard-Andersen O (1994). In: Tietz textbook of Clinical Chemistry (Burtis C.A. and Ashwood E.R. Ed.) W.B. Saunders Company London. pp. 1395-1406

[25] 25- Beuge, J. A., & Aust, S. D. (1978). Estimation of serum malondialdehyde level. Methods in enzymology Hoffee Jones edt. By Hoffee PA and Jone ME. Academic Press, a Subsidinary of Harcoart Brace Jovanovich Publisher, New York.

[26] 26- Wierusz-Wysocka, B., Wysocki, H., Byks, H., Zozulińska, D., Wykrętowicz, A., & Kaźmierczak, M. (1995). Metabolic control quality and free radical activity in diabetic patients. Diabetes research and clinical practice, 27(3), 193-197.

[27] 27- Opsomer, G., Mijten, P., Coryn, M., & de Kruif, A. (1996). Post-partum anoestrus in dairy cows: A review. Veterinary Quarterly, 18(2), 68-75.

[28] 28- Channo, A., Kaka, A., Kalwar, Q., Jamali, I., Jelani, G., Bakhsh, M., ... & Goil, J. P. (2022). An overview of bovine cystic ovarian disease. Pakistan Journal of Zoology, 54(5), 2437.

[29] 29- Ali, F., Lodhi, L. A., Hussain, R., & Sufyan, M. (2014). Oxidative status and some serum macro minerals during estrus, anestrous and repeat breeding in Cholistani cattle. Pak Vet J, 34(4), 532-534.

[30] 30- Perumal, P., De, A. K., Bhattacharya, D., Alyethodi, R. R., & Kundu, A. (2021). Effect of exogenous melatonin on endocrinological profiles, biochemical and antioxidant and

oxidative stress profiles in post partum anestrus Andaman local buffaloes in tropical island ecosystem. Indian Journal of Animal Sciences, 91(3), 206-213.

[31] 31- Mohebbi-Fani, M., Mirzaei, A., Nazifi, S., & Tabandeh, M. R. (2012). Oxidative status and antioxidant enzyme activities in erythrocytes from breeding and pregnant ewes grazing natural pastures in dry season. Revue de Médecine Vétérinaire, 163(10), 454-460.

[32] 32- Kono, Y., & Fridovich, I. (1982). Superoxide radical inhibits catalase. Journal of Biological Chemistry, 257(10), 5751-5754.

[33] 33- Gaweł, S., Wardas, M., Niedworok, E., & Wardas, P. (2004). Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci lekarskie (Warsaw, Poland: 1960), 57(9-10), 453-455.

[34] 34- Bartlewski, P. M., Beard, A. P., Cook, S. J., & Rawlings, N. C. (1998). Ovarian follicular dynamics during anoestrus in ewes. Reproduction, 113(2), 275-285.

[35] 35- Hafez, M. H. (2019). Serum Hormonal, Metabolic and Minerals Profile in Normal Cyclic and Postpartum Anestrus Egyptian Buffaloes. Alexandria Journal for Veterinary Sciences, 60(2).

[36] 36- Havern, R. L., Whisnant, C. S., & Goodman, R. L. (1994). Dopaminergic structures in the ovine hypothalamus mediating estradiol negative feedback in anestrous ewes. Endocrinology, 134(4), 1905-1914.

[37] 37- Lawania, P., Jingar, S. C., Roy, A. K., Kumar, D., & Kumar, A. (2017). Evaluation of Hormonal Treatment Protocol and Improved Nutritional Therapeutic Management Practices for Inducing Heat in Anestrus Cows. Int. JCurr. Micro. App. Sci, 6, 510-512.

[38] 38- Saleh, N., Mahmud, E., & Waded, E. (2011). Interactions between insulin like growth factor 1, thyroid hormones and blood energy metabolites in cattle with postpartum inactive ovaries. Nat. Sci, 9(5), 56-63.

[39] 39- Humphrey, W. D., Kaltenbach, C. C., Dunn, T. G., Koritnik, D. R., & Niswender, G. D. (1983). Characterization of hormonal patterns in the beef cow during postpartum anestrus. Journal of Animal Science, 56(2), 445-453.

[40] 40- Sathya, A., Prabhakar, S., Sangha, S. P. S., & Ghuman, S. P. S. (2007). Vitamin E and selenium supplementation reduces plasma cortisol and oxidative stress in dystocia-affected buffaloes. Veterinary research communications, 31, 809-818.