Research Article

Impact of Mastitis Incidence during Early Postpartum on Reproductive Efficiency, Antioxidant Status, Hormonal Profile, and Trace Elements in Lactating Egyptian Buffaloes

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Abstract | This study aimed to determine the influence of early postpartum clinical mastitis on the reproductive performance, antioxidant, hormonal profile and trace elements in Egyptian buffaloes. A total of 90 multiparous lactating Egyptian buffaloes (500-600 kg live body weight, 5-7 years old, 3-5 parities, and 10.75 ± 2.66 kg/day milk yield were used in this study. The buffaloes were managed and feed at similar traditional conditions. Mastitis diagnosis was performed 21 days after calving, then animals (n=90) were grouped to normal (n= 75), and clinical mastitis (n=15) groups. Results show that interval from calving to first estrus (31.60±0.86 vs. 33.10±0.98 d) or first service (55.20±0.97 vs. 61.00±1.96) was not affected (P≥0.05). Mastitis decreased (P<0.05) estrus/mating (93.3 vs. 80.0%) and pregnancy rates (86.66 vs. 53.33%), prolonged (P<0.001) days open (63.45±3.25 vs. 104.10±3.92 d), and increased services number required for conception (1.35±0.11 vs. 2.800±0.25 services). Mastitis decreased (P<0.0001) reduced glutathione (5.66±0.182 vs. 4.71±0.302 mmol/l) and total antioxidant capacity (1.64±0.046 vs. 0.76±0.049 mmol/l), and increased (P<0.0001) in malondialdehyde (0.80±0.043 vs. 1.68±0.064 mmol/ml). Mastitis significantly decreased serum concentration of progesterone (P4), estrogen (E2), thyroxin (T4), zinc (Zn), and selenium (Se) by about 24.05, 13.80, 5.36, 2.50, and 3.44%, respectively, while triiodothyronine (T3) concentration showed non-significant decrease by about 3.77%. Mastitis incidence at early postpartum in lactating Egyptian buffaloes delayed the resumption of estrous and ovarian activity and decreased pregnancy rate, antioxidant status, thyroid and reproductive hormones and trace elements such as Zn and Se.

Keywords | Mastitis, Buffaloes, Pregnancy rate, Antioxidant, Trace elements.

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INTRODUCTION

Livestock raising is a very important part of animal production including milk and meat production for human consumption (Zigo et al., 2019). Among livestock animals, buffaloes have economic importance for milk and meat production (Ahmed, 2006). In some developing countries, buffaloes are accounting for 12% of global milk production (Mansoor et al., 2017). In the buffalo population, mastitis is an important and persistent infection culminating in economic losses due to a drop in milk production, the treatment cost, and culling process (Dhakal and Thapa, 2002; Singh and Bansa, 2004).

In the dairy industry worldwide, mastitis is the most health disorder of cow herds. It causes several changes, physically, chemically and bacteriologically, in lactating buffalo milk leading to inferior changes in milk production (Sharma et al., 2007; Guccione et al., 2014), and increasing rate of culling and costs of treatment (Sharma et al., 2004; Ali et al., 2013). It is considered the major problem even though teat dipping in various antiseptic solutions and total dry cow therapy (Khokon et al., 2017). In dairy cows, economic loss of mastitis per animal estimated 695.7 EGP yearly, and cows after the fifth parity should be culled from the dairy herd, with high care during the winter season to keep the udder clean and dry and give the high producing cows immunostimulant drugs to reduce the incidence of mastitis (Abd-El Hamed et al., 2020). Estimating its economic losses could give the farmers and veterinarians a clear vision of the mastitis costs at the herd level to help in making appropriate decisions toward its control (Moru *et al*., 2018).

Reproductive efficiency plays a great role in the dairy industry as it is considered the most important factor affecting the profitability in dairy farms. Reproductive efficacy was closely related to uterine, claw and udder health status (Khalil and Hussein, 2019). In lactating cows, the health of the mammary gland was correlated with the fertility (Chebel et al., 2004). A negative correlation was found between the reproduction and incidence of clinical mastitis in cows leading to alteration in inter-estrus interval and decreasing the luteal phase (Moore et al., 1991), and poor pregnancy rate (Hertl et al., 2010). Also, the high incidence of mastitis was associated with increased postpartum first service interval, the number of services per conception, days from calving to conception, embryonic loss, abortion, and failure to become pregnant to the first service and decreased pregnancy rate (Moore et al., 2005; Pinedo et al., 2009; Lavon et al., 2011; Hudson et al., 2012).

Pathogens may impair ovulation, fertilization and loss of embryos in Holstein heifers (Peter et al., 2004). Incidence of clinical mastitis within the breeding period of crossbred cows had more negative impacts on reproductive measurements (Kumar et al., 2016). The negative impacts included a reduction in milk yield (Fleischer et al., 2001; Hansen et al., 2004) and decreasing reproductive efficiency (Hockett et al., 2000; Schrick et al., 2001). The prevalence of mastitis ranged between 10 and 50%; it is affected by different factors, such as sanitation, milk production, and animal breed (Sharma et al., 2004). In Egypt, clinical mastitis prevalence in lactating buffaloes was 12% (Ali Al-Zainy and Al-Jeburii, 2015).

The oxidative stress (OS) in relation to different diseases involving mastitis is a complex phenomenon. In living tissues, the free radical generation is a normal process. Under the normal condition, antioxidant defense system effectively counteracted the reactive oxygen species (ROS) by enzymatic or non-enzymatic antioxidants (Jozwik et al., 2012). The imbalance between ROS and antioxidants causes OS (Lykkesfeldt and Svendsen, 2007), leading to immunity dysfunction and increased inflammatory response (Abuelo et al., 2013), and enabling the microbial pathogens establishment in the mammary gland. The OS is induced by exceeding oxidant production over antioxidant defense capacity leading to macromolecular damage in lipids, proteins and DNA (Sordillo and Aitken, 2009).

Although buffalo has been traditionally considered less susceptible to mastitis than cattle, there are a little information concerning the effect of mastitis incidence in buffaloes on some reproductive, immunity, antioxidant, menial. Therefore, the goal of the present experiment was to evaluate the reproductive traits, blood antioxidant, hormonal profile and trace elements in healthy and mastitic Egyptian buffaloes before service.

MATERIALS AND METHODS

This study was conducted on 90 lactating Egyptian buffaloes taken from Mehallet Mousa Experimental Station in the North Nile Delta belongs to Animal Production Research Institute (APRI), Agricultural Research Center, Kafrelsheikh Governorate, Egypt.

Animals

In this experiment, 90 lactating Egyptian buffaloes (500-600 kg live body weight, 5-7 years old, having 3-5 parities, and 10.75 ± 2.66 kg/day milk yield were used in this study. Body condition score (BCS) of all buffaloes was performed according to Ezenwa et al. (2009). It ranged from 3 to 4 [scale from 1 (emaciated) to 5 (very fat)]. All animals were at early lactation (one- month postpartum).

All animals were maintained in semi-open pens. Animals were healthy after calving without problems (normal offspring and no retained placenta). The reproductive tract was observed by rectal palpation and transrectal ultrasonography scanning within the 1st month postpartum. Also, they did not suffer metabolic disorders during the postpartum period.

Feeding system

The buffaloes were kept under similar traditional feeding and managerial factors applied in the experimental station. Diets covering requirements of maintenance and milk yield were offered to buffaloes according to Animal Production Research Institute (APRI), Egypt. These diets were consisting of a clover (*Trifolium alexandrinum*, 2nd **Table 1:**

Chemical composition of the feedstuffs (on DM basis).

Feedstuff	Dry matter (%)	Chemical composition on DM basis (%)					
		Organic matter	Crude protein	Ether extract	Crude fiber	Nitrogen free extract	Ash
Concentrate feed mixture	90.09	89.7	16.7	4.6	18.6	49.00	11.1
Clover (2nd cut)	16.08	85.9	15.7	2.1	20.7	47.1	14.4
Clover (3rd cut)	20.07	85.8	14.7	3.0	22.4	46.2	13.7
Corn silage	38.14	91.40	10.94	4.33	18.84	57.29	8.60
Rice straw	93.05	82.9	3.57	1.66	36.4	42.00	16.1

and 3rd cuts), rice straw, and feed mixture (33% un-decorticated cottonseed, 23% corn, 22% wheat bran, 13% rice bran, 2% molasses, 3% limestone, 1.5% common salt and 2.5 % calcium). Animals were fed twice a day (7 a.m. and 6 p.m.), drinking water was available all-day times. Methods of AOAC (1990) were used for the chemical analyses of different feedstuffs (Table 1).

Mastitis diagnosis

Animals were milked by machine twice daily (6 a.m. and 5 p.m.). After 21 days of postpartum period, individual milk samples were collected by hand before milking for mastitis diagnosis as described by National Mastitis Council (2001). Wet udder hygiene with water was performed before milking to remove dirt from the udder and teats, then the udder is thoroughly wiped with wet wipes moistened with an antiseptic solution (Valiant-ABS CZ s.r.o.).

Udder secretions were collected and clinical data recorded from apparently healthy animals. The examination included an assessment of the general condition of the animal. California mastitis test (CMT) was used to diagnose mastitis in buffaloes. After the manual milking of each quarter of the udder into a dark-bottomed vessel, the reagent was mixed with the milk. Depending on the composition of the gel, after mixing the result was recorded as a negative, positive, or trace in each milk sample (Jackson and Cockcroft, 2002). The abnormality in milk such as presence of discoloration, water-like consistency, clots, flakes, and/or blood, along with udder inflammation (≥1 quarters) was defined mastitis.

Experimental design

According to mastitis diagnosis, buffaloes (n=90) were grouped to a normal group (healthy animals, n= 75) with negative clinical signs in milk and normal udders, and another group with clinical mastitis (n=15), in which milk has clinical signs (positive). These results indicated an incidence of mastitis in 15 out of 90 buffaloes, representing 16.67%.

As a routine work in the station, a mastitis test was monthly performed on all animals. Cases of mastitis in our study were discovered only at the early postpartum period (1st month). No clinical mastitis cases were diagnosed in the normal group thereafter.

Reproductive traits

At early postpartum, all animals were observed twice daily at an interval of 12 h by experienced herd man for at least one hour for estrus signs. Animals stand to be mounted by the teaser bull were considered in estrus. After 45-60 days of calving (voluntary waiting period before the first service), all experimental animals in heat were naturally mated with fertile buffalo bull according to the morning/evening system of breeding. On days 28-30 post-breeding, non-return animals were ultrasonography investigated to indicate pregnancy, while those returned to estrus were re-mated.

Postpartum 1st estrus (PPFEI) or mating (PPFMI) interval, mating rate (MR), number of services per conception (NSC), days open (DO), and pregnancy rate (PR) were recorded as reproductive traits of animals in the normal and mastitis groups.

The number of animals in heat (mated animals) was recorded, then mating and pregnancy rates were calculated. Mating rate = mated animals/total number of animals x100. Pregnancy rate = Number of conceived animals/total number of naturally-bred animals x100. Days open was determined as an interval from parturition to animals to conceive, then services number required for conception was calculated.

Blood sampling

On 0 and 10 days of the estrous cycle, blood samples were taken from 10 animals in each group when the animals showed estrus symptoms. Blood samples were collected from the *jugular vein* into a clean vacutainer tube free from anticoagulant. Blood samples were centrifuged (3000 rpm for 10 minutes) and clear serum free from hemolysis was separated.

Blood serum was stored at -20°C until analysis. In blood serum taken on Day 0 of the estrous cycle, antioxidant

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Table 2: Postpartum reproductive traits of HB and MB groups (data are expressed as mean ±SE).

Reproductive trait	Healthy Buffaloes (HB)	Mastitic Buffaloes (MB)	P-value(Sign.)	Overall Mean+
Postpartum first estrus interval (d)	31.60±0.86	33.10±0.98	0.294ns	32.10±0.66
Postpartum first mating interval (d)	55.20±0.97	61.00±1.96	0.006ns	57.13±1.03
Mating rate	70/75 (93.3)	12/15 (80.0)	*	82/90 (91.1)
Days open	63.45±3.25	104.10±3.92	0.0001***	77.00±4.34
Number of services/conception	1.35±0.11	2.800±0.25	0.0001***	1.83±0.16
Pregnancy rate	65/70 (92.85)	8/12 (66.66)	*	73/82 (89.0)
Pregnancy rate+	65/75 (86.66)	8/15 (53.33)	*	73/90 (81.1)

ns: Not significant. * Significant at $P < 0.05$. *** Significant at $P < 0.001$. + Based on the total number of buffaloes in HB and MB groups.

Table 3: Antioxidant status of buffaloes in HB and MB groups at estrus (data are expressed as mean ±SE).

Antioxidant marker	Healthy buffaloes (HB)	Mastitic Buffaloes (MB)	P-value(Sign.)	Overall Mean+
Glutathione reduced (mmol/l)	5.66±0.182a	4.71±0.302b	0.0001***	5.34±0.176
Total antioxidant capacity (mmol/l)	1.64±0.046a	0.76±0.049b	0.0001***	1.35±0.084
Malondialdehyde (mmol/ml)	0.80±0.043b	1.68±0.064a	0.0001***	1.39±0.089

*** Significant at $P < 0.001$. + Based on total number of buffaloes in HB and MB groups.

Table 4: Concentration of thyroid hormones, reproductive hormones, and trace elements in blood serum of buffaloes in HB and MB groups at estrus (data are expressed as mean ±SE).

Item	Healthy Buffaloes (HB)	Mastitic Buffaloes (MB)	P-Value(Sign.)	Overall Mean
Metabolic hormone:				
Ttriiodothyronine (ng/ml)	95.56±1.87	84.74±9.68	0.146ns	91.95±3.48
Thyroxine (µg/dl)	3.36±0.162	2.81±0.129	0.035*	3.18±0.124
Reproductive hormones:				
Estrogen (pg/ml)	20.76±0.554	16.46±0.427	0.0001***	17.90±0.503
Progesterone (ng/ml)+	5.28±0.205	1.48±0.125	0.0001***	4.01±0.361
Minerals (trace elements)				
Zinc (µg/dl)	131.17±1.35	121.35±1.14	0.0001***	127.89±1.29
Selenium (µg/dl)	136.65±1.47	122.55±1.29	0.0001***	131.95±1.62

+ On Day 10 of the estrous cycle. n.s.: Not significant * Significant at $P < 0.05$. *** Significant at $P < 0.001$.

parameters (total antioxidant capacity, TAC; reduced glutathione, GSH, and malondialdehyde, MDA), thyroid hormones (triiodothyronine, T3; thyroxine, T4), trace elements (zinc, Zn; selenium, Se) and estradiol (E2) were analyzed. On Day 10 of the estrous cycle, the concentration of serum progesterone (P4) was measured.

Analytical assays

The level of TAC was calorimetrically determined in blood serum after the method of Koracevic et al. (2001). The level of glutathione (GSH) and malondialdhyde (MAD) were assayed according to Yoshida et al. (2005). Concentrations of T3, T4, P4, and E2 in blood serum samples were assayed by direct radioimmunoassay technique (RIA) using specific chemical kits according to the procedures outlined by the manufacturer (Diagnostic Products Corporation, DCP Los Angles, USA). The serum concentration of selenium (Se) and zinc (Zn) was performed by atomic absorption spectrophotometer according to Varley et al. (1980).

Statistical analysis

T-test analysis was used for statistical analyzing the collected data using SAS software (SAS, 2004). Chi-square test was used to analyze the rate of estrus/mating and pregnancy rate. The formula given in the SAS software was used to calculate the interval of confidence for prevalence.

RESULTS

Reproductive traits

Results revealed that the interval from calving to first estrus (PPFEI) or first mating (PPFSI) were not affected significantly by clinical mastitis. However, the incidence of mastitis showed significantly deleterious effects on estrus/mating and pregnancy rates, days open, and number of services per conception (NSC) in mastitic buffaloes (MB) in comparison with healthy buffaloes (HB, Table 2).

Antioxidant activity

Regarding the level of different antioxidants activities (Table 3), there was a decrease (P<0.0001) in glutathione reduced (GSH) and total antioxidant capacity (TAC), and an increase (P<0.0001) in malondialdehyde (MDA) in MB as compared to HB group.

Hormonal profile and trace element contents

Regarding thyroid and steroid hormones, it has been observed that serum concentration of T4, E2 (on day of estrus), and P4 (on day 10 of the estrous cycle) were significantly increased in HB compared with MB, while there was a non-significant variation of T3 on day of estrus. Serum concentrations of Zn and Se on the day of estrus was significantly (P<0.01) higher in HB than in MB (Table 4).

DISCUSSION

Incidence of clinical mastitis within the breeding period is the main negative factor affecting milk yield of dairy cows. Beside this effect, there are several negative aspects of incidence of mastitis before service on the reproductive traits, blood antioxidant, hormonal profile and trace elements of Egyptian buffaloes, which is considered the aim of our study. The obtained results revealed that the negative impact of mastitis incidence (overall mean of total experimental animals) relative to healthy buffaloes by increasing days open (21.36%) and the number of services per conception (35.56%), and decreasing estrus/mating (2.36%) and pregnancy rates (6.42%) as illustrated in Figure 1.

In accordance with our results, several investigators mentioned that mastitis has been shown to harm reproduction in dairy cattle (Oliver et al., 2000; Schrick et al., 2001; Santos et al., 2004; Huszenicza et al., 2005). In dairy cows, clinical mastitis increased the insemination interval (85 vs. 61.5 d), insemination index (1.2 vs. ≥2.0, Zigo et al., 2019), and the duration of both PPFSI and days open (215±44.90 and 297±34.80 vs.78.88±3.86 and 133.32±12.90 d, Borpujari et al., 2019) as compared to controls. Also, Kumar et al. (2016) reported that clinical mastitis significantly (P<0.05) increased PPFEI, PPFMI, days open and NSC compared to clinically healthy cows. Also, delayed PPFEI (Vacek et al., 2007) and PPFSI (Schrick et al., 2001) in clinical mastitis affected animals before the first AI has been reported. In general, the pregnancy rate of cows reduced from 54.9 to 38.1% when clinical mastitis occurr pre-service (Yang et al., 2012) or from 38.71 to 61.39% (Bouamra et al., 2017), and increased PPFSI to 7-10 days (Linderoth, 2003) in comparison with non-mastitic cows. In buffaloes, the conception rate 40–45 days post-mating increased to 55% (P < 0.05) in controls as compared to 18.18% in mastitic animals (Mansour et al., 2017). On the other hand, the pregnancy rate on Days 25 and 45 after insemination was lower with clinical (28 and 16%) in comparison with those without mastitis (69.57%, P < 0.05) (Mansour et al., 2016). In other studies, (Hudson et al., 2012; Fuenzalida et al., 2015), clinical mastitis has been associated with low odd to pregnancy, especially when the case of mastitis is occurring near before or after the service or during the breeding period.

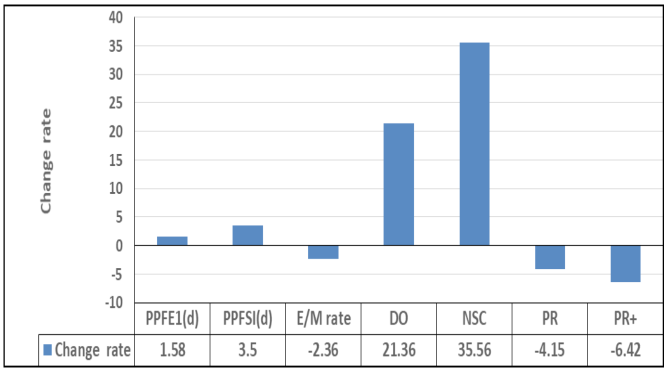

Figure 1: Effect of mastitis incidence on change rate (+/-) in different reproductive traits of buffaloes. (+ Based on the total number of buffaloes in HB and MB groups)

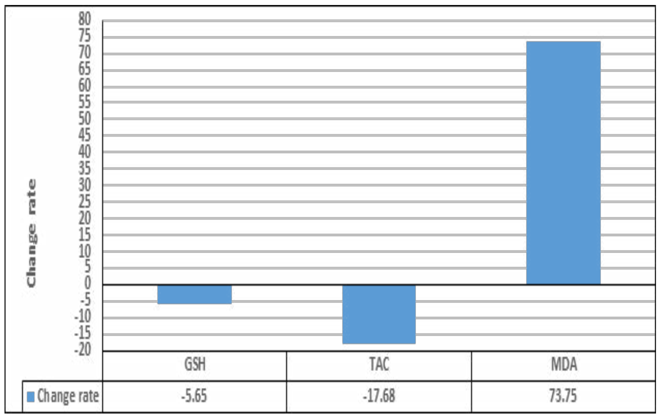

Figure 2: Effect of mastitis incidence on change rate (+/-) in serum antioxidants of buffaloes.

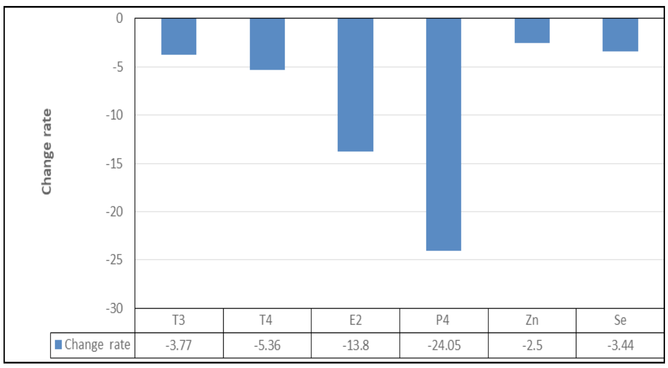

Figure 3: Effect of mastitis incidence on reduction rate in hormones and minerals in serum of buffaloes.

In dairy cows, fertility is depending on service interval, conception after the 1st service, insemination index, and the calving interval. The possible reason that mastitis has a negative effect on reproductive performance was attributed to reduction of gonadotropin supporting ovulation, in vivo maturation of oocytes, folliculogenesis, and function of CL. Also, Hansen et al. (2004) reported that mastitis may increase cytokines secretion, which can inhibit LH secretion and reduce blood P4 level as well as an alteration in hypothalamic-pituitary hormonal axis. In addition, mastitis may cause a rise in lipopolysaccharide and PGF2α levels which deleteriously affecting function of oocyte or development of embryo (Soto et al., 2003).

In buffaloes, the incidence of mastitis causes a reduction in the diameter of preovulatory follicles at estrus (Mansour et al., 2017), and suppression of diameter and function of CL, resulting in increasing embryonic mortality rates (Mansour et al., 2016). Premature luteolysis and consequent loss of P4 and thus early embryonic mortality (Huszenicza et al., 1998) may delay the onset of ovarian activity and estrus in MB group. Moreover, the PGF2α production which causes CL regression may be increased by Gram-negative mastitis pathogens leading to embryonic mortality (Moore and O'Connor, 1993). Gram-positive and -negative bacterial pathogens increased the interval from calving to 1st service, days open, and the number of services required for conception (Barker et al., 1998).

As such we also observed that the highest rate of change in antioxidant parameters by mastitis incidence was in terms of increasing MDA level by about 73.75% and decreasing both TAC and GSH levels by 17.68 and 5.65%, respectively (Figure 2). These results revealed an association of increased MDA level as a marker of lipid peroxidation with mastitic buffaloes. In dairy cows, there are various factors (genetic, physiological, and environmental) affecting the host and pathogens that can compromise host immunity and increase mastitis incidence (Blowey and Edmondson, 2010; Zigo et al., 2017). In dairy cows, the disturbance in the homeostasis by generation and accumulation of ROS, resulted in oxidative stress leading to mastitis incidence (Poławska et al., 2012). The mastitis incidence increased by impairing the immunity and antioxidant status of the udder (Zigo et al., 2019). The present results concerning oxidative stress markers and enzymatic antioxidants activities indicated a reduction in TAC and GSH levels along with an increase in MDA level in MB in comparison with HB. Similarly, in cows with mastitis, there was a significant decrease in TAC and a significant increase in MDA (Mohamed et al., 2017), a significant reduction in GSH level (Jhambh et al., 2013) as compared to the control group. However, an enhanced GSH concentration in plasma has been demonstrated in cows with mastitis by Kizil et al. (2007) with the explanation that there might be enhanced activities of GSH dependent enzymes leading to intense regeneration of GSH from GSSH.

Malondialdehyde, the lipid peroxidation end product, is one of the most reliable and often used indexes of oxidative stress (Kapusta et al., 2018). In mastitic cows, the concentration of MDA in milk was considered as a marker of the reduction in milk yield (Sharma et al., 2011) because MDA concentration was higher in the sub-mastitis milk than in the normal milk, and could be considered as an indicator of the sub-mastitis udders (Zigo, et al., 2019). The level of MDA increased in the milk of mastitic cows infected with staphylococci or streptococci (Suriyasathaporn et al., 2012). A significant increase in erythrocytic MDA production in the cows with clinical mastitis was reported as compared to healthy control (Jhambh et al., 2013). Also, an enhanced plasma MDA concentration has been documented in cows with clinical mastitis (Kizil et al., 2007).

In addition, marked reduction in hormonal profile and trace elements occurred by mastitis, being 3.77, 5.36, 13.8, and 24.05% for T3, T4, E2, and P4, and 2.5 and 3.44% in Zn and SE, respectively (Figure 3). The impaired reproductive traits observed in MB in association with a significant reduction in T4 and insignificant decrease in T3 level may be due to the importance of the thyroid hormones for animal reproduction. In lactating buffaloes, subtle thyroid activity may have some effects on fertility (Aggarwal and Singh, 2010; Ghuman et al., 2011). In this way, Mutinati *et al.* (2010) reported that ovarian function in cattle was directly affected by stimulatory effects of the thyroid hormones. At the same time, thyroid hormones were found to regulate ovarian steroidogenesis and low T3 level was in relation to reducing the levels of estradiol and decreased estrus expression (Jorritsma et al., 2003). This finding was observed in our study by decreasing both E2 and thyroid hormones. In cattle, T3 and T4 have an important effect on the development of embryos before and after implantation (Ashkar et al., 2010). Induced hypothyroidism was known to reduce the fertilization rate in dairy cattle (Bernal et al., 1996). Several authors (Malinowski and Gajewski, 2010; Wolfenson et al., 2015; Mansour et al., 2016) mentioned that mastitis increased PGF2α level and possibly TNFα levels related to CL regression, decreased P4 level and then pregnancy rates. During mastitis, increasing cytokines such as TNF-β and IFN-δ was found to be cytotoxic to the CL and may decrease level of P4 (Petroff et al., 2001).

The observed higher conception rate in HB than in MB may be related to the increase in P4 concentration observed on day 10 post-service (luteal phase). Likewise, P4 concentrations on day 9 through 25 after AI were greater ($P < 0.05$) in healthy cows as compared to subclinical mastitis and clinical mastitis cows, and mastitis incidence revealed suppression to both CL diameter and function leading to a significant reduction in pregnancy outcome of buffalo cows (Mansour et al., 2016; 2017). The preovulatory follicles are found for increasing circulating E2 levels at estrus, and was positively correlated with preovulatory follicles diameters (Pandey et al., 2011; Rahman et al., 2012a, b). Therefore, occurring the mastitis pre-AI (during the follicular phase) reduced the pulsatile secretion of LH, leading to low E2 secretion close to estrus, and delayed surge of LH and consequently occurring the ovulation (Hockett et al., 2005; Lavon et al., 2008).

In accordance with the present results, serum Zn concentration increased with increasing ovarian activity in cyclic buffaloes. During estrous cycle, Zn concentration was the lowest in pro-and met-oestrous periods (Alavi-Shoushtari et al., 2015). In anestrus Nili-Ravi buffaloes, there was a significant reduction in serum Zn and Se concentrations as compared to cyclic ones (Akhtar et al., 2009). The reduction in Zn level was in relation to decreasing concentration of steroid hormones. There are correlations between blood Zn concentration and each of P4 and E2 concentrations. Generally, the Se deficiency resulted in a marked reduction in fertility (Hidiroglou, 1979) and low Se levels decreased the anestrus incidence (Harrison et al., 1984). These findings were observed in our study explaining the relationship between each mineral contents (Zn and Se), hormonal profile (P4, E2, T3 and T4) with fertility, specifically in mastitic buffaloes. Minerals, such as Zn and Se, have long been recognized as antioxidants in animal health and production (Yang and Li, 2014), having a specific role in mastitis of dairy cows. Zn increased leukocyte function, and increased susceptibility to bacterial infection, while Se increased efficiency in neutrophils function, and improved bactericidal capabilities of neutrophils, and both decreased severity and duration of mastitis (Hayajneh, 2014).

CONCLUSION

Incidence of clinical mastitis at early postpartum in lactating Egyptian buffaloes has deleterious effects on reproductive performance in terms of delayed postpartum first service interval, increased days open, number of services per conception, and decreased pregnancy rate. Besides the known reduction in milk yield of mastitic animals, there is a relationship between the incidence of mastitis and antioxidant defiance system, thyroid hormones (Thyroxin) and trace elements such as Zn and Se.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

NOVELTY STATEMENT

Authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. There has been no significant financial support for this work that could have influenced its outcome. As Corresponding Author, I confirm that the manuscript has been read and approved for publication by all the named authors.

AUTHOR CONTRIBUTIONS

Substantial contributions to conception and design (A.E. Abdel-Khalek and M.A. Abo-Farw), acquisition of data (M.A. Abo-Farw and M.A. Aboul-Omran), analysis and interpretation of data (A.E. Abdel-Khalek and M.A. Abo-Farw), statistical analyses (M.A. Abo-Farw and A.E. Abdel-Khalek), drafting the manuscript (M.A. Abo-Farw, A.E.; Abdel-Khalek and M.A. Aboul-Omran), critically revising the manuscript for important intellectual content (A.E. Abdel-Khalek and M.A. Abo- Farw). All authors contributed to conducting research and writing this manuscript.

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