REPRODUCTIVE PERFORMANCE OF EGYPTIAN BUFFALO BULLS ADMINISTRATED WITH N-ACETYLCYSTEINE, L-CARNITINE, OR THEIR COMBINATION

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SUMMERY

This study was to evaluate the effect of daily oral administration of N-acetylcysteine (NAC), L-carnitine (L-C), or their Combination for 2 months pre-semen collection on semen quality, sexual desire and some blood parameters traits of Egyptian buffalo bulls. A total of 20 bulls (400±37 kg LBW and 24-25 mo old) were divide into 4 groups. In group1 (G1, control), G2, G3 and G4, animals fed a basal ration and the managed under the same conditions, and were received orally a daily dose of 0, 3 L-C, 3 g NAC, or 1.5 g NAC +1.5 g L-C as a combination/bull/day, respectively, for 2 months. The results showed that bulls that received the combination dose (G4) increased ($P<0.05$) the concentration of total proteins, albumin, globulin and glucose, while it decreased ($P<0.05$) total cholesterol, triglyceride, creatinene concentrations, and AST and ALT activities compared with the control and other groups. All physical characteristics in fresh and thawed semen were higher ($P<0.05$) in all treated groups than in the control group, being the best in G4. Plasma testosterone and total antioxidant capacity concentrations were increased ($P<0.05$) in G4, G3 and G2 compare with the control (G1), being the best for G4 bulls. In conclusion, an oral dose of 1.5 g NAC plus 1.5 g L-C bull/day, as a combination, for 2 months pre-semen collection improves health status, immunity response and antioxidant capacity of buffalo bulls to produce semen with high quality require for spreading the tool of artificial insemination in buffaloes.

Keywords: *Buffaloes, N-acetylcysteine, L-carnitine, semen quality, blood and testosterone.*

INTRODUCTION

All over the world, buffaloes are economic farm animals for the production of milk and meat. There is a need for researches to improve several considerations for the importance of buffalo (Michelizzi *et al*., 2010). Male is a factor remaining a common reason of infertility; thus, it important to accurate assessment of semen quality with improving buffalo bull semen cryopreservation for animal breeding. Buffalo semen with good quality is require for natural breeding and artificial insemination (AI) to increase the reproductive efficiency of buffalo females (Wafa *et al*., 2017).

Oxidative stress (OS) has been identified as a major mediator in various etiologies of male infertility, the release of harmful decay products such as reactive oxygen species (ROS) can increases defective spermatozoa that led to decreasing the total number of fertilization-competent spermatozoa in AI semen doses (Thundathil *et al*., 2001; Aitken *et al*., 2006).Therefore, gains in sperm viability and a reduction of the minimum necessary sperm number per AI dose could be achieved by the supplementation antioxidants and by the development of simple, rapid, and inexpensive techniques for the removal of defective spermatozoa (Petruska *et al*., 2014). The treatments of oxidative stress, including oral antioxidants, have been widely studied in patients with infertility caused by reactive oxygen species (ROS) directly or indirectly (Agarwal *et al*., 2014).

Low molecular weight compounds such as N-acetylcysteine (NAC) and carnitine are protective antioxidant system comprehends, enzymatic, non-enzymatic factors which interact each other to ensure an optimal protection against the oxidative stress. A deficiency in one of these antioxidants may result in a decrease of total plasma antioxidant capacity (Walczak-Jedrzejowska *et al*., 2013). NAC is a nucleophilic amino acid that is converted in the body to cysteine, a precursor to glutathione which is the main endogenous antioxidant against lipid peroxidation in the epididymis and testes (Mora-Esteves and Shin, 2013). NAC exerts its antioxidant actions essentially by two mechanisms. It is capable of easily penetrating cellular membranes and, once inside the cells. Although extensive research has been made in the antioxidant protective effects of NAC on ROS-induced side effects (Lanzafame *et al*., 2009; De la Cruz Rodriguez et al., 2010; Wang *et al*., 2014), the amount of studies focused on male fertility is scarce. NAC has free radical scavenging activity both *in vivo* (Ciftci *et al*., 2009) and *in vitro* (Hussein, 2018). Carnitine is a high-polarity natural compound, similar to vitamins-like amino acids. Within the body of most animals, it is synthesized from lysine and methionine. It is a water soluble antioxidant, especially in its forms L-carnitine (Vaz and Wanders, 2002). High concentrations of carnitine are present in the male reproductive tract and particularly in the epididymis, suggesting its crucial role in energy metabolism and sperm maturation (Ng *et al*., 2004). L-carnitine (L-C) involves in sperm motility as it is considered a source of energy, and it is acts as an essential co-factor for the transport of long chain fatty acids within the mitochondrial matrix in order to facilitate the oxidative processes and to enhance cellular energy production(Agarwal and Said, 2004).

Daily treatment with NAC results in a significant improvement in sperm function by reducing ROS and oxidation of sperm DNA (Safarinejad and Safarinejad, 2009; Jannatifar *et al*., 2019). Also, oral treatment with NAC (600 mg daily) was associated with significant improvement in sperm motility with idiopathic infertility in human (Ciftci *et al*., 2009). Friesian bulls, high-quality semen was produce by oral dose of 2 g/h/ L-C for 2 months (Abdel-Khalek *et al*., 2015), and increased sperm motility and pregnancy rate in human (Lenzi *et al*., 2004; Balercia et al., 2005). Also, addition of L-C on semen extender of cryopreserved semen improves sperm activity (Banihani *et al*., 2014; Hussein, 2018).

Therefore, this study aimed to the impact of NAC, L-C or their combination as oral dose for 2 months pre-semen collection on sexual desire, semen production, blood biochemicals and hematological parameters, antioxidant and health statues of Egyptian buffaloe.

MATERIALS AND METHODS

The experimental work of this study was done at Animal Production Research Institute (APRI), Egypt, in cooperation with Department of Animal Production, Faculty of Agriculture, Tanta University.

Animals and experimental design*:*

A total of twenty healthy Egyptian buffalo bulls weighing 400±37.5 kg and 24-25 months old were divided to four experimental groups according to age and body weight (5 bulls in each). The control bulls received a basal diet without treatments (G1). Each bull in G2, G3, and G4 receive the control diet with daily oral-dose of 3 g L-C, 3 g NAC and 1.5 g NAC+1.5 g L-C for 2 months a treatment period, respectively. The experimental bulls were kept under the same conations of housing (individually in semi-open sheds), environment and management.

Feeding system:

The experimental bulls in all groups receive individual feeding on a diet containing concentrate feed mixture (CFM), berseem hay (BH) and rice straw (RS) according to APRI buffalo bull requirements. Feeds were offered at 7 a.m. and 4 p.m., while drinking water was offered all day time. Ingredients and chemical analysis of different feedstuffs are presented in Table (1).

Table (1): Chemical analysis of CFM, BH an RS in the basal ration of the experimental bulls.

Item	DM	Chemical analysis (%, on DM basis)					
		OM	CP	EE	CF	NFE	ASH
Concentrate feed mixture	91.50	88.74	15.85	4.70	13.66	54.53	11.26
Rice straw	92.30	79.63	3.47	1.41	35.10	39.65	20.37
Berseem hay	89.00	85.96	15.96	2.92	28.2	38.88	14.04

DM= Dry matter, OM= Organic matter, CP= Crude protein, CF= Crude fiber, EE= Ether extract

Semen collection:

After a treatment period of two months with different types of antioxidants, semen was collected twice a week using an artificial vagina (IMV, France) for 2 months (collection semen period). The collected semen of each group was individually placed in a water bath (37^{o}C), and then taken immediately to the laboratory for evaluation of fresh semen. During the collection period, the reaction time (RT) was recorded in term of time elapsed from exposing each bull to a suitable stimulus and the first copulation.

At the last week of the collection period, one half of each ejaculate after evaluation was centrifuged at 3000 rpm for 15 minutes at room temperature to obtain the individual samples of the seminal plasma (Khan *et al*., 2015**)**, which stored at -20ºC until analyses. The second half of the ejaculates was prepared for freezing process.

Semen cryopreservation:

The collected ejaculates of all bulls in each group were pooled per collection day, then semen was diluted by Tris-base extender (TEY) with pH value of 6.8 and osmolarity level of 280-300 mOsm/l at a rate of 1:10 (semen: extender). The TEY contained 3.63 g tris, 0.5 g fructose, 1.99 g citric acid, 100 mg streptomycin, and 100.000 IU penicillin dissolved in 100 ml distilled water. In 83 ml of TEY extender, egg yolk (10 ml) and glycerol (7 ml) were added. After cooling the diluted semen at 5^{o}C for 4 h as an equilibration period, semen was aspired into 0.25 ml French straws, then straws were sealed using polyvinyl alcohol powder and expose for 10 min at 5 cm above liquid nitrogen (LN) vapor, then plunged into LN (-196^{o}C), stored one month. During evaluation of semen, the straws were warmed at 37^{O}C for 30 second in a water bath.

Evaluation of semen:

Percentages of progressive motility, livability, abnormality, and acrosomal status of sperm cell were performed according to Amman and Hammerstedt (1980), Hackett and Macpherson (1965), Blom (1983), and Yanagimachi (1982), respectively, in fresh and thawed semen. Also, sperm cell concentration per ml was determined to calculate total sperm output/ejaculate. Sperm cell concentration/ml (SCC), was estimsted by Haemocytometer (Khan, 1994). Total sperm output/ejaculate (TSO) was compute using the following formula: *TSO = Ejaculate volume (ml) x SCC/ ml.* Total antioxidant concentration was also determined in post-thawed semen according to Koracevic et al. (2001).

Blood samples:

At the end of experiment, blood samples were taken before morning feeding from animals in each group via the jugular vein into test tubes containing heparin (anticoagulant). Each blood samples were divided to 2 portions; the first was centrifuged at 3000 rpm for 15 minutes to separate blood plasma, while the second were prepared for hematological parameters.

Blood plasma samples were kept at -20^{o}C till determination of total proteins (Tietz, 1990) and albumin (Tietz, 1994) concentrations. Globulin concentration was obtained by subtracting the concentration of albumin form the total proteins. Concentrations of triglycerides, total cholesterol,

creatinine, and glucose in blood plasma were also determined according to Mc Gowan *et al*. (1983), Richmond (1973), Bartles *et al*. (1972), Trinder (1969) according to Reitman and Frankal (1957), activity of asprtate (AST) and alanine (ALT) aminotransferases in blood plasma was determined.

Concentrations of testosterone (Ekins, 1984) and total antioxidants (Koracevic *et al*., 2001) in blood plasma were determined. Plasma biochemicals in plasma were calorimetrically determined using spectrophotometer and by commercial kits (diagnostic system laboratories, INC, USA).

Blood hematology involved counts of erythrocyte (RBCs) and luekocytes (WBCs) were counted using Haemocytometer, while haemoglobin (Hb) concentration and hematocrit (PCV %) were directly measured according to Henry (2001) by commercial kits (Mission® Plus, REF C132-3031, USA).

Statistical analysis:

The obtained results were statistically analyzed by SPSS (2013) to study the effect of treatment on different variables using one way-ANOVA. The significant mean differences were set by Duncan Multiple Range Test (Duncan, 1955) at $P<0.05$.

RESULTS AND DISCUSSION

Semen production and sperm output:

Results presented in Table (2) showed that the sexual desire in term of reaction time and all semen-quality parameters of buffalo bulls were improved significantly ($P<0.05$) in treatment groups G2, G3, and G2 in comparing with untreated-control group (G1). The maximal beneficial effects were recorded in G4 for buffalo bulls treated with L-C and NAC combination.

Table (2): Effect of L-carnitine (L-C), N-acetylcysteine (NAC), or their combination on sexual desire and semen parameters in buffalo bulls.

Semen trait	Experimental group			
	G1 (Control)	G2 (LC)	G3 (NAC)	G4 (LC+NAC)
Reaction time (second)	95.83±1.08^{a}	67.78±0.89^{b}	66.63±0.90^{b}	59.03±0.65^{c}
Ejaculate volume, ml	2.12±0.09^{c}	2.59±0.09^{b}	2.63±1.00^{b}	3.21±0.08^{a}
Progressive sperm motility, %	66.67±0.50^{c}	72.53±0.89^{b}	72.78±0.88^{b}	77.72±1.11^{a}
Live sperm, %	68.58±0.67^{c}	74.08±0.88^{b}	75.00±0.85^{b}	78.83±1.08^{a}
Abnormal sperm, %	26.05±0.39^{a}	17.70±0.72^{b}	17.42±0.74^{b}	13.67±0.98^{c}
Acrosomal damage, %	25.28±0.41^{a}	19.60±0.40^{b}	19.43±0.39^{b}	14.58±0.66^{c}
Sperm concentration, x10^{9}/ml	0.848±0.04^{c}	1.098±0.03^{b}	1.251±0.07^{a}	1.350±0.05^{a}
Total sperm output, x10^{9}/ejac.	1.809±0.13^{c}	2.913±0.18^{b}	3.371±0.29^{b}	4.381±0.26^{a}

a, b, and c: Group differences within each row at P<0.05.

In comparing our result with other authors, the dietary addition of L-C improved semen quality (Jacyno *et al.,* 2007), and increased the ejaculate volume and concentration of spermatozoa in boar (Wahrner *et al.,* 2004). Dietary L-C supplementation increased ejaculate volume and sperm viability of boar (Akey, 2000). Generally, previous studies suggested that male infertility may be treated by carnitine (Matalliotakis *et al*., 2000; Lenzi *et al*., 2003). The L-C plays an important role as an antioxidant. It increased number of sperm cells (Zhai *et al*., 2007) as well as progressive sperm motility and vitality in infertile males (Vicari and Calogero, 2001) via preventing the lipid peroxidation by decreasing ROS production patients**.**

On the other hand, NAC is protective antioxidant system comprehend acts as protectors of oxidative stress. It improves the total plasma antioxidant capacity (Walczak-Jedrzejowska *et al*., 2013) against lipid peroxidation in the epididymal and testicular tissues (Mora-Esteves and Shin, 2013) and protecting from ROS-induced side effects (Wang *et al*., 2014). It was reported that NAC has *in vivo* (Ciftci *et al*., 2009) and *in vitro* (Hussein, 2018) free radical scavenging activity. In similar pattern, NAC and L-C act as key role in sperm metabolism. They proved readily available energy for sperm cell activity that has a positive

effect on sperm mobility (Matalliotakis *et al*., 2000). A secondary role of NAC and L-C as antioxidants is counteracting and eliminating several body oxidation factors and protecting the normality and physiological function of body cells (Dokmeci, 2005).

Characteristics of the seminal plasma:

It was observed that total cholesterol (TC), triglycerides (TG) concentrations, and AST and ALT activity significantly (P<0.05) decreased, while level of total antioxidant capacity significantly (P<0.05) increased in seminal plasma of bulls by all treatments. Treatment of bulls with L-C and NAC combination showed significantly (P<0.05) the highest positive impact on lipid profile, membrane integrity and antioxidant status of the seminal plasma of buffalo bulls (Table 3).

Table (3): Effect of L-carnitine (L-C), N-acetylcysteine (NAC), or their combination on chemical composition, enzyme activity, and total antioxidant capacity of the seminal plasma of fresh buffalo semen.

Item	Experimental group			
	G1 (Control)	G2 (LC)	G3 (NAC)	G4 (LC+NAC)
Seminal plasma biochemical				
Total cholesterol, g/dl	96.89±2.35^{a}	88.97±2.26^{b}	84.31±1.99^{bc}	80.34±1.71^{c}
Triglyceride, g/dl	46.32±1.02^{a}	39.21±1.13^{b}	37.65±0.97^{b}	33.86±0.76^{c}
Enzyme activity in seminal plasma:				
AST, IU/l	57.78±1.35^{a}	46.71±1.13^{b}	45.32±1.12^{b}	40.29±1.94^{c}
ALT, IU/l	26.34±0.81^{a}	22.41±0.47^{b}	21.05±0.51^{b}	18.84±0.31^{c}
Total Antioxidant in seminal plasma:				
TAC (mM/l)	1.75±0.18^{c}	2.63±0.19^{b}	2.88±0.18^{b}	3.63±0.18^{a}

a, b, and c: Group differences within each row at P<0.05.

Activity of transaminases AST and ALT in the seminal plasma is used as an indicator of sperm membrane integrity because releasing sperm enzymes into the seminal plasma was found to be in association with injury in spermatozoa (Rasul *et al*., 1999). Therefore, decreasing the activity of AST and ALT in the seminal plasma of all treatment groups, especially those treated with L-C and NAC combination may indicate intact plasma membrane, normality and viable sperm cells (Daader *et al*., 1993; Abdel-Gawad *et al*., 2000). Similar results were reported regarding the effect of treatment on antioxidant status of the seminal plasma, In this context, using *Moringa oleifera* leaves (MOL), as a natural antioxidant, significantly decreased serum TC and TG concentrations in buffalo bulls, and increased activity of catalase, glutathione (GSH) and SOD as antioxidant enzymes (Wafa *et al*., 2017). In rabbits, the MOL extract could decrease concentrations of TC and TG (Chumark *et al*., 2008). Lipid peroxidation in blood was decreased in rabbit bucks by MOL extract (El-Harairy et al., 2016). This may be due to the high content of flavonoids in MOL (Asma *et al*., 2005).

Sperm freezing ability:

Data presented in Table (4) clear that all sperm characteristics in thawed semen of buffalo bulls including percentages of progressive motility, livability, abnormality, acrosomal damage of sperm cells as well as TAC level showed significant (P<0.05) improvement with G2 an G3 in comparing with G1.

Table (4): Effect of L-carnitine (L-C), N-acetylcysteine (NAC), or their combination on freezing ability and total antioxidant capacity of buffalo semen.

Item	Experimental group			
	G1 (Control)	G2 (LC)	G3 (NAC)	G4 (LC+NAC)
Sperm characteristics in thawed semen:				
Progressive sperm motility, %	45.15±0.66^{d}	57.95±0.50^{c}	59.85±0.64^{b}	63.10±0.57^{a}
Live sperm, %	47.00±0.70^{c}	58.95±0.80^{b}	60.35±0.68^{b}	63.90±0.84^{a}
Abnormal sperm, %	48.90±0.67^{a}	27.30±0.56^{b}	26.60±0.37^{b}	19.70±0.47^{c}
Acrosomal damage, %	49.00±0.79^{a}	31.70±0.87^{b}	30.50±0.62^{b}	22.70±0.50^{c}
Total antioxidant capacity (TAC) of the thawed seminal plasma:				
TAC (mM/l)	2.87±0.24^{b}	3.10±0.23^{ab}	3.22±0.23^{ab}	3.75±0.29^{a}

a, b, and c: Group differences within each row at P<0.05.

Buffalo bulls treated with L-C and NAC combination showed the highest benefits on freezing ability of cryopreserved semen, which was in harmony with semen physical characteristics of the same bulls in fresh semen.

The obtained results are in accordance with those reported on the impact of antioxidant supplementation in semen extender during cryopreservation by different species. In buffalo bulls, El-Sheltawi *et al*. (1999) showed that post-thawing motility was proved to be higher than control after *in vivo* and/or *in vitro* supplementation of vitamin E. El-Siefy (2004) demonstrated that post-thaw progressive motility after 30 days of deep freezing was improved in bulls treated with selenium plus vitamin E. El-Hawary (2010) showed that sperm characteristics were proved to be higher (P<.0.0) in post-thawed semen of buffalo bulls treated with vitamin E + Zinc than control. In rams, Gokcen *et al*. (1990) indicated that rams supplemented with different antioxidant were significantly better in the frozen semen characteristics and acrosomal morphology of spermatozoa than that of non- supplemented rams.

Biochemicals, enzyme activity, testosterone profile and total antioxidant capacity in blood plasma:

The concentrations of biochemicals including total proteins (TP), albumin (AL), globulin (GL), and glucose increased (P<0.05), while total cholesterol, triglycerides and creatinine decreased (P<0.05) in blood plasma of bulls with groups G2, G3 and G4 than control group. In addition, AST and ALT activities reduced (P<0.05), however, testosterone and TAC levels were improved (P<0.05) in blood plasma in all treatment groups. Generally, among all treatments L-C and NAC combination reflected the highest improvement on protein and carbohydrate metabolism, lipid profile, kidney and liver function, antioxidant defense system, and sexual desire of buffalo bulls (Table 5).

Table (5): Effect of L-carnitine (L-C), N-acetylcysteine (NAC), or their combination some biochemicals, enzyme activity, testosterone profile and total antioxidant capacity in blood plasma of buffalo bulls.

Item	Experimental group			
	G1 (Control)	G2 (LC)	G3 (NAC)	G4 (LC+NAC)
Blood biochemicals:				
Total proteins, g/dl	7.24±0.24^{c}	8.14±0.25^{b}	8.48±0.25^{b}	9.46±0.37^{a}
Albumin, g/dl	3.01±0.18^{b}	3.42±0.17^{b}	3.50±0.15^{b}	4.62±0.37^{a}
Globulin, g/dl	4.23±0.16^{b}	4.73±0.18^{ab}	4.97±0.17^{a}	4.83±0.22^{a}
Total cholesterol, g/dl	172.04±5.26^{a}	149.42±4.13^{b}	149.59±2.57^{b}	134.84±2.14^{c}
Triglycerides, g/dl	60.44±1.55^{a}	53.04±1.50^{b}	53.62±1.82^{b}	49.48±1.39^{b}
Creatinine, mg/dl	0.88±0.02^{a}	0.71±0.04^{b}	0.68±0.03^{b}	0.62±0.04^{b}
Glucose, mg/dl	57.82±1.54^{c}	61.55±0.46 ^{b}	63.57±0.85^{ab}	66.19±0.80^{a}
Enzyme activity:				
AST, IU/l	59.03±1.25^{a}	51.45±1.53^{b}	50.54±1.18^{b}	46.79±0.85^{c}
ALT, IU/l	23.74±0.49^{a}	18.51±0.37^{b}	17.15±0.36^{c}	15.68±0.32^{d}
Testosterone and total antioxidant capacity (TAC):				
Testosterone (ng/ml)	5.12±0.07^{d}	6.65±0.06^{c}	6.97±0.07^{b}	7.59±0.09^{a}
TAC (mM/l)	2.33±0.13^{c}	3.48±0.18^{b}	3.92±0.17^{b}	4.59±0.23^{a}

a, b, and c: Group differences within each row at P<0.05.

These results may be in association with the antioxidant properties of L-C, NAC or the synergic effects of their combination on antioxidant defense system by protecting body cells from the harmful effects of ROS due to lipid peroxidation. In this respect, many authors found a significant positive effect of antioxidants (MOL), as a dietary supplement, on increasing the concentration of TP, AL, GL, glucose, testosterone and TAC, and reducing total cholesterol, triglycerides, and creatinine concentrations as well as AST and ALT activities in blood of buffalo bulls (Wafa *et al*., 2017) and rabbit bucks (El-Ratel, 2017). Supplementation of L-C significantly increased glucose concentration in blood of rabbits (Chapa *et al*., 2001). However, NAC treatment was found to improve biochemical parameters in serum, decrease the hepatoxicity, in term of reducing activity of AST and ALT of Wistar male rats severed from toxicity (Turkmen *et al*., 2019). The hepatoprotective effects of NAC may be attributed to that NAC supplementation resulting attenuation of liver damage (Lasram *et al*., 2014). The indicated hepatoprotective effect of NAC might be attributed to the homeostasis in the oxidant/antioxidant status supplied by NAC (Turkmen *et al*., 2019). In our study, the NAC alone or in combination with L-carnitine

showed protection against oxidative stress injury to keep the structural integrity of hepatic cells preventing release of intracellular enzymes into the blood (Izadia *et al*. 2011).

The noted association between testosterone concentration and TAC was reported by Jannatifar *et al*. (2019). This may reflect that all treatments, in particular their combination, had beneficial effect lipid peroxidation and oxidative stress index (Agarwal and Majzoub, 2017). Therefore, the improvement observed in fresh semen of all treatment groups was mainly due to improving the concentration of testosterone and TAC levels.

Based on the present results, treatment with L-C, NAC, or their combination had no effect on liver and kidney function, but positively affected antioxidant defense mechanism (Asal Lamiaa, 2013).

Hematological parameters:

Results concerning hematological parameters of buffalo bulls as shown in Table (6) revealed that count of red blood cells (RBCs), hemoglobin concentration (Hb) and packed cell volume (PCV) showed significant ($P<0.05$) improvement in treatment groups G4, G3 and G2 compared with G1. Count of WBCs decreased significantly ($P<0.05$) in G2 and G4 compared with G1. Treatment with L-C and NAC combination showed the best impact on all hematological parameters of buffalo bulls, exhibiting synergetic effect of L-C and NAC on blood hematology of buffalo bulls. Similar results indicated improvement of hematological parameters of buffalo bulls treated with MOL (Wafa *et al*., 2017), and the Nubian Sudanese goats fed on MOL (Babeker and Abdalbagi, 2015) as antioxidants. In general agreement with the present results, natural antioxidant administrations have beneficial effects on hematological parameters of different species, including the impact of green tea extract on rabbit does (El-Ratel *et al*., 2017), rabbit bucks treated with lycopene (El-Ratel, 2017), rabbit bucks treated with propolis (Hashem et al., 2013), impact of MOL extract on rabbit bucks (Chumark *et al*., 2008; Chinwe and Isitua, 2010; Ahemen *et al*., 2013).

Table (6): Effect of L-carnitine (L-C), N-acetylcysteine (NAC), or their combination on some hematological parameters in buffalo bulls.

Item	Experimental group			
	G1 (Control)	G2 (LC)	G3 (NAC)	G4 (LC+NAC)
RBC ($x10^6/mm^3$)	6.43 ± 0.19^{c}	7.44 ± 0.17^{b}	7.53 ± 0.13^{b}	8.27 ± 0.15^{a}
WBC ($x10^3/mm^3$)	7.73 ± 0.18^{a}	7.10 ± 0.14^{bc}	7.46 ± 0.15^{ab}	6.97 ± 0.15^{c}
Hb (mg/dl)	7.92 ± 0.25^{c}	9.19 ± 0.27^{b}	9.12 ± 0.23^{b}	10.36 ± 0.28^{a}
PCV (%)	31.44 ± 0.51^{c}	33.59 ± 0.26^{b}	33.83 ± 0.25^{ab}	34.71 ± 0.17^{a}

a, b, and c: Group differences within each row at P<0.05. RBC= Red blood cells, WBC=White blood cells, Hb=Hemoglobin, PCV=Packed cell volume.

In the presence of unchecked ROS accumulation, hematopoietic cells appear to be particularly vulnerable, because the deficiency in antioxidant defense system leads to either anemia and/or hematopoietic tissues malignancies (Kong *et al*., 2004). Therefore, the improvement observed in hematological parameters of buffalo bulls by treatment of L-C, NAC or their combination in our study may be related to the impact of these treatments as antioxidants by protecting against ROS generation in haematopoietic cells.

CONCLUSION

In conclusion, oral dose of 1.5 g NAC plus 1.5 g L-C bull/day, as a combination, for 2 months pre-semen collection improves health status, immunity response and antioxidant capacity of buffalo bulls to achieve high quality semen for spreading the artificial insemination with buffalo semen with high fertility.

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الأداء التناسلي لفحول الجاموس المصري المعامل ب أن-أستيل سيستين، إل-كارنيتين أو كليهما

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كان الهدف من هذه الدراسة هو تقييم تأثير المعاملة بـ أن- استيل سيستين، ال- كارنتين أو خليط بينهما عن طريق الفم لمدة شهرين قبل جمع السائل المنوي على جودة السائل المنوي والرغبة الجنسية وبعض سمات مقاييس الدم لفحول الجاموس المصري. تم تقسيم 20 فحل (400 ± 37 كجم وزن الجسم وعمر24-25 شهرًا) إلى اربع مجموعات. جميعها غذيت علي عليقة المحطة، المجموعة الاولي كنترول بدون معاملة، المجموعة الثانية، الثالثة والرابعة عوملوا بـ 3 جرام من أن- استيل سستين و ال - كارنتين و بـ 1.5 جرام أن- استيل سيستين + 1.5 جرام ال - كارنتين رأس/يوم/فحل علي التوالي. أظهرت النتائج أن الفحول في المجموعة الرابعة زادت زيادة معنوية ($P<0.05$) في تركيز البروتينات الكلية، الألبومين، الجلوبيولين والجلوكوز، بينما انخفض تركيز كل من الكوليسترول الكلي، الدهون الثلاثية، الكرياتينين ونشاط الانزيمات (AST و ALT) مقارنة مع مجموعة الكنترول والمجموعات الأخرى. تحسنت جميع خصائص السائل المنوي الطازج والمذاب ($P<0.05$) في جميع المجموعات المعاملة مقارنة بمجموعة الكنترول ، حيث كانت المجموعة الرابعة أفضل ($P<0.05$) مقارنة مع المجموعة الثانية والثالثة وكان تركيز هرمون التستوستيرون وتركيز مضادات الأكسدة الكلي أعلى معنويا ($P<0.05$) في المجموعات المعاملة مقارنة بمجموعة الكنترول، حيث كانت المجموعة الرابعة هي الأفضل.

يستنتج من الدراسة ان المعاملة عن طريق الفم بـ 1.5 جرام أن- استيل سيستين + 1.5 جرام ال - كارنتين رأس/يوم لفحول الجاموس المصري لمدة شهرين قبل جمع السائل المنوي يحسن من الحالة الصحية ، والاستجابة المناعية وقدرة مضادات الأكسدة لإنتاج السائل المنوي بجودة عالية المستخدم في التلقيح الاصطناعي في الجاموس لتحسين الاداء التناسلي.