PROTECTIVE EFFECT OF N-ACEETYLCYSTEIN AGAINST OXIDATIVE STRESS IN RELATION TO SEMEN CHARACTERISTICS IN NEW ZEALAND WHITE RABBITS

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SUMMARY

A total of 15 mature New Zealand White (NZW) rabbit bucks, aged 6-8 months, weighing about 3.5 Kg were divided into three equal groups, control group (G1) that injected subcutaneously with saline solution, while bucks in G2 and G3 were injected subcutaneously with 50 and 100 mg NAC / kg body weight, respectively for seven consecutive days. Blood samples were collected at days 8and day 47, and semen characteristics were evaluated at day 48. The results revealed that there was a positive effect of NAC on antioxidant status through its obvious impact on MDA level that continuously decreased, in G3, from 6.10 to 3.58 nmol/ml, from zero day-to-day 47.While, G1 recorded continuous increase. Additionally, NAC injection with the dose of 100 mg/kg BW succeeded in elevating catalase activity that elevated from 329.8 to 372.4 U/L) on the 8th day compared to its levels in zero day. Moreover, H_2O_2 was insignificantly declined and this decline continued until the day 47 in G3 (declined from 0.093, at zero day to 0.083 mmol/L, at day 47) with stable level in G2 (0.098 mmol/L, at 8th and 47 days). No side effects of the NAC injections were detected as confirmed by normal activity of liver enzymes (ALT and AST) in rabbits. The same trend was noticed for kidney functions. Additionally, an enhancement of semen characteristics due to NAC injections was observed, especially in G3.

Keywords: Rabbit, bucks, N-acetylcysteine, Antioxidants, liver and kidney functions, semen characteristics

INTRODUCTION

Oxidative stress, OS (exhaustion of antioxidant regulation), can simply defined as the imbalance in redox system resulting from producing high levels of reactive oxygen species (ROS). Reproduction is one of the biological functions that requires extra energy for its activity, which expected to cause generation of ROS. Testes are already producing high level of ROS, and considered more vulnerable than other tissues to OS, which lead to cell damage. Accordingly, the intrinsic factors such as the incidence of sperm abnormalities (dead sperm and immature spermatozoa) may cause an increase in OS (Pintus and Ros-Santaella, 2021). The generated ROS can attack spermatozoa causing loss of cell membrane function. This is due to, the higher presidency of polyunsaturated fatty acids (PUFA) in spermatozoa cell membrane, who are very sensitive to lipids peroxidation (Garcia *et al*., 2011). Besides that, the extrinsic factors like climate change, the way of sperm handling and storage are also associated with increasing ROS production that impair fertility(Pintus and Ros-Santaella, 2021). Using antioxidants can eliminate the negative impact of OS on male fertility; one of these antioxidantsis N-acetylcysteine (NAC).As previously proved by Mohammadi-Sardoo *et al*. (2018) who stated that, NAC had protective effect on male rat testes from fungicides, by modifying ROS expression.

The NAC has developed since1960s;it is a commercial product, available in pharmacies, its main use is for human respiratory diseases. Actually, most of studies dealt with NAC from a therapeutic point of view. Such as, Cam *et al*., (2008), who tested NAC in rabbits, to treat them from aflatoxins (AF), using higher doses 250 and 500 mg NAC /kg body weight (BW) for five days. They reported that, NAC succeeded in reducing liver damage caused by AF compared to untreated group, and these doses of NAC did not caused any side effects. Several approaches for improving reproductive and productive performance of rabbits by using different antioxidant materials, but utilizing NAC was rare. To date, may be the only available research work was conducted by Ashour *et al.* (2018).

Therefore, the present study was executed to examine and evaluate the protective effect of N-acetylcystein on health status including antioxidant markers, liver and kidney functions. In addition, to assess the semen characteristics of NZW rabbits during breeding season in Egypt.

MATERIALS and METHODS

Ethical approval:

The experimental work was carried out in the Rabbitry Unit located in Laboratory of Animal Physiology, Faculty of Agriculture, Cairo University in cooperation with Animal Production Research Institute (APRI), Agricultural Research Center (ARC). All experimental procedures had been conducted according to ethical rules of both aforementioned governmental entities.

Place of work and animals:

The experimental work was carried out in the rabbitry Unit located in Laboratory of Animal Physiology, Faculty of Agriculture, Cairo University.

Fifteen mature NZW rabbit bucks aged between 6-8 months with an average of body weight (BW) 3521±18.40g were used in this experiment. Bucks were in a good health and no signs of abnormalities were observed (Photo, 1). All bucks were kept in semi-closed wire cages under the same managerial conditions. Rabbits were fed commercial pellet diets that satisfied their nutritional requirements according to the recommendations of APRI. These diets provided 18% crude protein, 13.4% crude fiber and digestible energy of 2500 kcal/ kg diet and having free water access.

The animals were divided into three equal groups (five bucks in each group). The first one considered as control group (G1) that injected subcutaneously with saline solution. The other two groups G2 and G3 were injected subcutaneously with 50 and 100 mg NAC/kg body weight (BW), respectively. The injections were lasted for seven consecutive days based on San-Migual *et al.,* 2006. The whole experiment was lasted for 96 days continuously during breeding season in Egypt starting from January to beginning of April.

Blood sampling and analysis:

Before starting saline and NAC injections (zero day), blood samples (3 ml) were collected from each buck in all groups from marginal ear vein in heparinized tubes. Then, the samples were centrifuged to separate blood plasma and kept frozen under 20°C until analysis. Following that, blood samples were collected at days 8th (after end of NAC injection by one day) and on day 47 (before semen collection by one day), (Fig., 1). The frozen plasma was used to determine antioxidant biomarkers including total antioxidant capacity (TAC, mmol/L), Malondialdehyde (MDA, nmol/mL), catalase (CAT, U/L) and Hydrogen peroxide (H_2O_2 mmol/L).

Activity of liver enzymes, aspartate aminotransferase (AST, U/L) and alanine aminotransferase (ALT, U/L) were determined as indicator of liver functions. Whereas, kidney functions, both concentration (mg/dl) of blood urea nitrogen (BUN) and creatinine (CR) were also quantified. All the analyzed parameters had been done using kits supplied from Bio-Diagnostic Company, Dokki, Giza, Egypt. All analytical steps were executed according to the procedures outlined by the manufacturer.

Photo 1. New Zealand White rabbit bucks.

Semen processing:

Bucks were well trained for semen collection using mature doe before starting the experiment. Semen was collected twice a week, in the morning at 8 a.m., from each buck in each group. The collection applied using artificial vagina (AV) and rabbit doe that inserted to buck cage until complete ejaculation

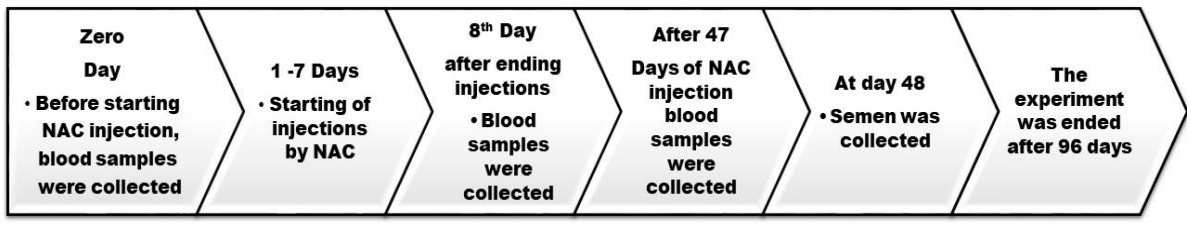

Figure 1. Experimental design of injected NZW rabbit bucks with NAC.

Semen characteristics:

Ejaculate volume and sperm cell concentration:

Ejaculates (n = 15, 5 bucks in each group) were pooled to avoid individual animal differences. Then semen volume had been calculated using graduated insulin syringe after removing gel mass. After that, semen was maintained in water bath (37°C) and transfer to laboratory with special attention and handling from direct light or heat shock (cold or hot) to evaluate semen characteristics. Haemocytometer was used to determine the sperm cell concentration (10^6/ml) according to the method described by Sorensen (1979).

Plasma membrane integrity:

Plasma membrane integrity (%) was determined using Hypo-Osmotic Swelling Test (HOST)

according to the method described by Jeyendran *et al.* (1984). A minimum of 200 sperm were checked under phase contrast microscopy (400x). Sperm storage displaying different types of swelling (coiling of tails) were considered as positive for the HOST. Percentage of hypo osmotic sperm swelling was calculated as the total number of spermatozoa with coiled tails / total number of sperm (coiled and normal tails) X 100.

Assessment of acrosome status:

Assessment of acrosome status of spermatozoa was executed according to the procedure outlined by Watson (1975). The percentage of intact acrosome was calculated for 200 spermatozoa selected randomly from each slide as number of intact spermatozoa / total number of checked spermatozoa X 100. Integrity status of spermatozoa was classified into three categories: intact (when the stain was clearly distributed over the anterior part of the sperm head to the equatorial segment), partially damaged (when the stain partially covered the acrosome region) and completely damaged (sperm with acrosome completely lost) when the sperm had no acrosome.

Sperm motility:

Sperm motility for control and treated groups were evaluated by CASA system (SpermVision™ software minitube Hauptstraße 41. 84184 Tiefenbach, Germany). The motion characterization was recorded including: distance curved line (DCL, µm), distance average path (DAP, µm), distance straight line (DSL, µm), velocity curved line (VCL, µm/sec), velocity average path (VAP, µm/sec), velocity straight line (VSL, µm/sec), linearity (LIN=VSL/VCL), straightness (STR=VSL/VAP), wobble (WOB=VAP/VCL), beat cross frequency (BCF, Hz) and amplitude of lateral head displacement (ALH, µm). the analysis was carried out by diluting 20 µl of semen with 280 µl of rabbit semen extender (consisted of 250 mM tris-hydroxy methylaminomethane, 83 mM citric acid and 50 mM glucose).

Statistical analysis:

The collected data were subjected to two way analysis of variance to detect the effects of treatment(T) and time of collecting blood samples (TBS) and their interaction (T*TBS) using the general linear model (GLM) procedure of SAS, 1999.

The statistical model used was as follows:

$Y_{ijk}= \mu + T_i + TBS_j+(T*TBS)_{ij} + e_{ijk}$

Where: Y_{ijk}= the individual observation, µ = The overall mean, T_i= The fixed effect of the i^{th} treatments (i= 1,2,3, where 1= G1; 2= G2, 3= G3), TBS_j = the fixed effect of the j^{th} time of blood samples (j=1, 2, 3, where 1= zero day, 2= the 8^{th} day, 3= day of 47), (T*TBS) ij = Effect of interaction between i^{th} and j^{th} (ij =1,……..9), e_{ijk}= Random error associated with the individual.

The collected data of CASA parameters and semen characteristics were subjected to one-way analysis of variance to detect the effect of treatment with NAC.

The statistical model used was as follows:

$Y_{ij}= \mu + T_i + e_{ij}$

The differences among treatments, time and interaction means were separated according to Duncan's Multiple Range Test (Duncan, 1955). The significance level was set at 5%.

RESULTS and DISCUSSION

Antioxidant biomarkers:

Total antioxidant capacity:

There were insignificant differences among all groups either affected by T of TBS or by their interaction as shown in Table (1). However, the treated groups (G3 followed by G2) were slightly higher in TAC values than those in G1.

Regarding the effect of NAC doses T and TBS, which showed that all values were in close to each other in all TBS. In addition, the interaction between the two factors (T and TBS) revealed that, G3 have noticeable increase in TAC level at day 47. It was higher than that its value in pre-and post NAC injection by 23%. Bucks in G2 showed lower (14.2%) TAC value at the day of 47 than that recorded at the 8^{th} day. However, in G1, TAC had higher level by 26.4% at day 47 than that at 8^{th} day.

Actually, TAC can be defined as, the ability of a compound to inhibit oxidative degradation of lipids that started with formation of conjugated dienes and triense, known as primary oxidation product due to abstraction of hydrogen atom (Chaiyasit *et al*., 2007). Therefore, increasing TAC values are valuable and important indicatorthat reflect the reduction in OS in blood (Abdelnour *et al*.,2021).

The present data were higher than that reported by El-Hammady *et al.*,(2017), who found that TAC level was 0.66 and 0.57 mmol/L in V-line and Moshtohor rabbits, respectively. The same trend was found by Abdelnour *et al*. (2021), who recorded 0.82 mmol/L for TAC in APRI rabbits. These differences may be attributed to the treatment they used or breed differences or management conditions.

Malondialdehyde:

Values of MDA in G2 were obviously lower ($P<0.05$) than those in G1 and G2, whereas the differences between G1 and G3were insignificant (Table 1)

In G1, levels of MDA were higher ($P<0.05$) than that in G2 by 66.8% and insignificantly than that in G3 by 3.9%. This result indicates that, the injectable dose of 50 mg NAC/kg BW was more effective in reducing MDA concentration than G3 (Table 1).

Table 1. Total antioxidant capacity(TAC) and malondialdehyde (MDA) levels in blood plasma of NZW rabbit bucks as affected by N-acetylcysteine injection

Items	TAC mmol/L			MDA nmol/mL		
Treatment (T, NAC doses)						
G1	0.97			5.09^{a}		
G2	1.06			3.05^{b}		
G3	1.11			4.9^{a}		
S.E	0.07			0.43		
Time of collecting blood samples (TBS)						
Day zero	1.03			4.43		
Day 8	1.01			4.29		
Day 47	1.11			4.42		
S.E	0.07			0.43		
Interaction (T ×TBS)						
	G1	**G2**	**G3**	**G1**	**G2**	**G3**
Day zero	0.97	1.10	1.03	4.27^{abc}	2.90^{bc}	6.10^{a}
Day 8	0.87	1.13	1.03	4.86^{ab}	2.69^{c}	5.30^{ab}
Day 47	1.10	0.97	1.27	6.13^{a}	3.55^{bc}	3.58^{bc}
S.E	0.12	0.12	0.12	0.75	0.75	0.75

[a, b,c] Means in the same column with different superscripts are significantly different (P<0.05). G1= control group; G2 and G3 = groups injected with 50 and 100 mg N-acetylcysteine (NAC) / kg body weight, respectively.

Results of TBS revealed that, values of MDA were almost close to each other in the three times. Meanwhile, the interaction revealed an important result, in which, MDA was very high before NAC injection (zero day) in G3 compared to the other two groups. It reached to 6.10 nmol/mL and then decreased at the 8thday by 13%. Then, continued in decreasing (P<0.05) at day 47, reached to 3.58 nmol/mL. This finding cleared the efficient role of 100 mg NAC/kg BW in declining level of MDA from zero day to day 47 by 41.3%. In comparison with G2, level of MDA concentration was reduced only from zero day to the 8th day by 7.2% only, then returned again to be higher (3.55 nmol/mL) at the day 47 than its level at zero day. These results confirmed the capability of the injectable dose100 mg NAC/kg in reducing MDA level, the main product of lipid peroxidation that occurs due to OS, than the dose of 50mg/kg BW. Moreover, MDA level in G1 was in continuing elevation and recorded its highest level (6.13 nmol/mL) at the day 47. Owing to the effect of TBS, values of MDA in G1 showed opposite trend to those in G3. The values in G1 were increased ascendantly with advancement of time, whereas those in G3 were decreased deascendantly with advancement of time (Table, 1). The current results are completely in accordance with Ashour *et al.* (2018), who concluded that, the dose of 100 mg/kg BW is more efficient in supporting antioxidant status in rabbits.

Catalase activity and levels of hydrogen peroxide:

Injection of NAC caused an increasing in CAT activity insignificantly in treated bucks (G2 and G3) than those in G1. CAT values in G3 that over passed G2 and G1 by 6% and 15.5%, respectively. Also, G2 had more CAT activity than that in G1 by 8.9%. These results agree with those of Eraslan *et al*. (2005) and Wang *et al*. (2015), who stated that, NAC is a protective element against oxidative damage through its favorable effect on CAT activity. It is well established that, CAT is the key enzyme in providing cell self-defense against H_2O_2, through using iron and manganese as cofactors in catalyzing H_2O_2 to H_2O and O_2. Actually, CAT has a great ability in breaking down million of H_2O_2 molecules that formed during oxidation of fatty acids. Regardless theeffect of T, CAT activity was lower at zero day than that at the 8th day by 15.4 %, with a little reduction in its activity at the day 47. The interaction between T and TBS was strongly cleared the positive role of NAC on CAT activity that elevated especially in G3, from 329.8 to 372.4 U/L at the 8th day compared to its levels in zero day, and higher than that in G1 and G2. Nevertheless, at day 47, CAT activity declined to be less than that recorded at the 8th day in G3, while it had a continuance elevation in G2, recording higher level (378.6 U/L) than the other groups. Whilst,G1 showed opposite trend, which recorded continuance decline in CAT activity from zero day to the day 47.

Therefore, this positive impact of NAC on CAT activity was reflected on the levels of H_2O_2,that significantly declined in treated groups (G2 and G3) than in G1.The decline rate is elevated with increasing NAC dose (G3, which recorded the lowest value for H_2O_2) as shown in Table (2). The interaction between T and TBS donated that, after injecting the experimental groups with NAC, H_2O_2 was insignificantly declined, and this decline continued until the day 47 in G3 with stable level in G2. On the other hand, in G1, level of H_2O_2 was significantly elevated after injecting with saline; this elevation may be attributed to the stress of injection. However, on day 47, H_2O_2 recorded its lowest value (0.087 mmol/L) in comparison with its level at zero and 8th days. Our findings are in accordance with those of Ashour *et al.* (2018) who also confirmed the protective role of NAC against OS. The aforementioned results, confirmed the ability of NAC, especially the dose of 100 mg NAC/ kg BW in improving antioxidant status in rabbit bucks. Due to its positive impact on TAC and reducing MDA

levels. Moreover, enhancing activity of CAT thus reducing levels of H_2O_2.

Table 2. Catalase (CAT)activity and hydrogen peroxide (H_2O_2) levels in blood plasma of NZW rabbit bucks as affected by N-acetylcysteine injection

Items	CAT (U/L)			H_2O_2(mmol/L)		
Treatment (T, NAC doses)						
G1	309.04			0.116^{a}		
G2	336.59			0.100^{b}		
G3	357.04			0.087^{c}		
S.E	31.69			0.004		
Time of collecting blood samples (TBS)						
Day zero	308.5			0.111^{a}		
Day 8	364.7			0.103^{a}		
Day 47	329.5			0.089^{b}		
S.E	31.7			0.004		
Interaction (T ×TBS)						
	G1	**G2**	**G3**	**G1**	**G2**	**G3**
Day zero	397.3	306.6	329.8	0.112^{b}	0.104^{bc}	0.093^{bcd}
Day 8	288.9	324.4	372.4	0.150^{a}	0.098^{bcd}	0.086^{cd}
Day 47	240.9	378.6	368.9	0.087^{cd}	0.098^{bcd}	0.083^{d}
S.E	54.9	54.9	54.9	0.006	0.006	0.006

[a, b,c] Means in the same column with different superscripts are significantly different (P<0.05). G1= control group; G2 and G3 = groups injected with 50 and 100 mg N-acetylecysteine (NAC) / kg body weight, respectively.

Liver enzymes:

The injectable dose of 100 mg/kg BW markedly reduced (P<0.05) ALT activity as in G3 than those in G1 and G2, which showed insignificant difference between them. The obtained values of ALT activity in G3was within normal range (6-9 U/L) as reported by Özkan *et al*. (2012), while in G1 and G2, ALT activity was exceeded the upper limit of recommended range. The effect of TBS recorded a reduction (P<0.05) in ALT activity by 33% at the 8th day. However, at day 47, ALT activity was elevated to higher value than that on the 8th day. This was much obvious in interaction between T and TBS that revealed insignificant decline in activity of ALT in G2 and G3 at 8th day, while there was a significant reduction in G1.Then, ALT activity increased in G1 and G3 before starting semen collection by one day (day 47) than that in 8th day. The opposite trend was noticed in G2 that showed further decline in ALT activity on the day 47.

With regard to the effect of T, AST activity showed the same trend of ALT. Both enzymes activity ascertained that, high dose of NAC (100 mg /kg BW) was more effective than that of low dose (50 mg /kg BW) in reducing liver enzymes (Table 3).

All AST values were within normal range (10-98 U/L) according to Verga (2002). The lowest value of AST activity was noticed in G3 that significantly (P<0.05) differed than G2 and insignificantly differed than G1. Concerning the effect of TBS, the allover mean of AST showed lower value (26.33 U/L) after ending NAC injection by one day. Then elevated on day 47. Actually, the interaction between T and TBS showed fluctuation in AST activity in times of pre – and post NAC injection. While at day 47, AST values were higher than the two other periods (pre and post NAC injection). The present data are in harmony with Ashour *et al*. (2018) who illustrated the safety of using NAC on liver enzymes in rabbits.

In 2006, San-Migual *et al*. (2006) used rabbits as an animal model to test the ability of NAC to treat hepatic failure. They injected the animal daily for 7 days with 150 mg NAC / kg BW. These rabbits were infected with hemorrhagic disease virus (RHDV, which cause hepatic damage). They found that NAC prevent the abnormal increases in ALT and AST in injected rabbits than the un-injected ones. They attributed that to, NAC is blocking apoptosis induced by cytotoxic agents and protecting liver cells from damage. So, from their study and the present data, NAC has no side effect on liver enzymes (ALT and AST, an indicator for liver cells damage). Additionally, all values of AST and ALT (in the current study),are fallen in normal range and did not reach to the upper limits of their normal range.

Kidney functions:

There were no significant differences in both BUN and CR concentrations in control (G1) and the other treated groups (G2 and G3) by the two doses of NAC. This indicated that, NAC injections had no harmful effects on kidney functions as ascertained by the obtained normal physiological values in this study.

As shown in Table (4), all the obtained values of BUN were within normal range (36.84 – 50.28 mg/dl) as stated by Verga (2002). In G1 and G2, BUN values were almost similar to each other, while G3 showed a slight elevation in BUN concentration (42.62 mg/dl) than the other two groups. Furthermore, data of TBS revealed a slight significant elevation in BUN at the day 47 than its level at zero day. The interaction T and TBS showed insignificant increase in BUN in G1 and G2 at the 8th day than that found in zero day. While G3, showed stable levels in the same periods. Likewise, a significant increase in BUN at day 47 was also observed inG1 and G3in comparison with its levels

pre-injections. Earlier, Ashour *et al*. (2018) pointed out to the factors affecting BUN. For instance, quality of feeds, protein concentration in the diet and feed restriction. The present data are in close to that reported by Ashour *et al*. (2018) who used two doses of NAC (50 and 100 mg/kg). They supported our current study and confirmed that NAC injection did not cause any abnormal elevations in BUN and may have positive impact on renal functions through excreting urea in urine.

Concerning CR, its values were fallen within normal range (0.5-2.5 mg/dl) as reported by Verga (2002). As for treatment effect, G3 recorded insignificant increase than G1 and G2. Regardless groups, TBS cleared that, NAC reduced CR concentration by 5.7% at the 8th day.

But, on the day 47, CR retained to increase reached to 1.94 mg/dl. The interaction between NAC doses and TBS clarified that, CR in G3 declined by 20% at the 8th day than that in zero day. The opposite was noticed in G2, NAC injection caused elevation in CR concentration by 35.6%, but still within normal range, than that recorded before one day of the treatment. In the present study, CR was approaching to the upper limit of normal range may not be caused by NAC injection. Because, Cam *et al*. (2008)used higher NAC (250 and 500 mg/kg BW) doses that intramuscularly injected in rabbits than we used, and found that CR level was ranged between 0.57 – 0.73 mg/dl, which did not reach to upper physiological limits. Also, the same results were found by Atef *et al*. (2016). Additionally, Ashour *et al*. (2018) who used same doses as we used in the present study and recorded that CR was ranged from 0.88 to 0.91 mg/dl.

Table 3. Activities (U/L) of alanine amino transaminase (ALT) and aspartate aminotransaminase (AST) and) in blood plasma of NZW rabbit bucks as affected by N-acetylcysteine injection

Items	ALT			AST		
Treatment (T, NAC doses)						
G1	11.00^{a}			27.83^{ab}		
G2	10.67^{a}			30.00^{a}		
G3	8.67^{b}			25.67^{b}		
S.E	0.62			0.92		
Time of collecting blood samples (TBS)						
Day zero	12.00^{a}			27.17^{b}		
Day 8	8.00^{b}			26.33^{b}		
Day 47	10.33^{a}			30.00^{a}		
S.E	0.62			0.92		
interaction (T × TBS)						
	G1	**G2**	**G3**	**G1**	**G2**	**G3**
Day zero	13.0^{a}	13.0^{a}	10.0^{ab}	29.5^{b}	26.5^{b}	25.5^{b}
Day 8	7.00^{b}	10.0^{ab}	7.00^{b}	25.0^{b}	28.5^{b}	25.5^{b}
Day 47	13.0^{a}	9.00^{b}	9.00^{b}	29.0^{b}	35.0^{a}	26.0^{b}
S.E	1.07	1.07	1.07	1.6	1.6	1.6

$^{a, b,c}$ Means in the same column with different superscripts are significantly different (P<0.05). G1= control group; G2 and G3 = groups injected with 50 and 100 mg N-acetylcysteine (NAC) / kg body weight, respectively.

Table4. Blood urea nitrogen (BUN) and creatinine (CR) concentrations (mg/dl) in blood plasma of NZW rabbit male as affected by N-acetylcysteine injection

Items	BUN			CR		
Treatment (T, NAC doses)						
G1	41.85			1.86		
G2	41.48			1.84		
G3	42.62			2.02		
S.E	0.62			0.12		
Time of collecting blood samples (TBS)						
Day zero	40.57^{b}			1.94		
Day 8	42.03^{ab}			1.83		
Day 47	43.35^{a}			1.94		
S.E	0.62			0.12		
Interaction (T × TBS)						
	G1	**G2**	**G3**	**G1**	**G2**	**G3**
Day zero	39.25^{c}	41.00^{bc}	41.45^{bc}	2.07	1.57	2.20
Day 8	42.85^{abc}	42.00^{abc}	41.25^{bc}	1.60	2.13	1.76
Day 47	43.45^{ab}	41.45^{bc}	45.15^{a}	1.90	1.83	2.10
S.E	1.07	1.07	1.07	0.21	0.21	0.21

$^{a, b,c}$ Means in the same column with different superscripts are significantly different (P<0.05). G1=control group; G2 and G3= groups injected with 50 and 100 mg N-acetylecysteine (NAC) / kg body weight, respectively.

Semen characteristics:

Tables (5) and (6) clarified the enhancement in most of semen characteristics and motility due to NAC injection, especially with the dose of 100 mg NAC/Kg BW.

The G3 has shown the noticeable improvement in semen volume, they produced by 7% and 22 % ml more semen than G1 and G2, respectively. In all groups, semen volume is considered normal, and at the optimum level for rabbits. McNitt *et al*. (2013) stated that, semen volume in NZW bucks is ranged between 0.4 -1.5 ml with an average 0.7 ml. So, the recorded value for semen volume in G2 do not considered as abnormal, but it is lower than the G1 and G3.

Taken together, semen samples of G3 contained much spermatozoa concentration (415×10^6/ml, Table, 5) than that in the other groups, especially with G2 that recorded the lowest value of spermatozoa concentration. This indicates that, the dose of 100 mg NAC /Kg BW may help testis to produce more semen volume with standard spermatozoa concentration. Interestingly, rabbit testis can produce up to 300 $\times10^6$/ml (150 $\times10^6$/ml, in average). This amount of spermatozoa is depending on breed, if bucks were recently used and stimulation level for semen collection. Bucks in all groups of this study, produced more spermatozoa concentration than the above mentioned value. This is indicating to the suitable environmental and managerial conditions, beside the effect of NAC treatment, that were provided for these bucks.

In the current study, plasma membrane intactness obtained the highest percentage in G3 followed by G2 and both are insignificantly slightly higher than that in G1. Definitely, plasma membrane intactness is highly correlated with sperm motility (will discuss later). Because, any damage in plasma membrane will result in rapid leakage of intracellular ATP that required to maintain sperm motility (Kordan *et al*., 1998).

In the same context, Ducci *et al*. (2002) illustrated that plasma membrane integrity is important to sustain sperm viability and functions. Because, this membrane is responsible in transporting the essential compounds that are required for sperm to perform its functions. Same results have been tabulated for acrosomal integrity, which was higher in treated groups than that in G1. Moreover, G3 obtained the best lower percentage in partial damage in comparison with the other groups. Whilst, G2 was the best in having lower sperm complete damage when compared with G1 and G3 (Table 5).

The quantitative CASA analysis of sperm motility as presented in Table (6) showed the significant effect of 100 mg NAC /Kg BW in improving sperm total motility and progressive motility. Additionally an observed increasing in sperm distance parameters (DAP, DCL, DSL) and in sperm fertilizing ability (velocity parameters; VSL, VAP, VCL) compared with the corresponding groups.

Inspection of spermatozoa motility (total and progressive motility) showed increasing trend ($P<0.05$) in G2 and G3 when compared to G1, and the highest motility(%)parameters were observed in G3. With respect to distance parameters (DAP, DCL, DSL), these parameters were significantly elevated with increasing the injectable dose of NAC. The groups of G2 and G3 were higher ($P<0.05$) in their distance parameters than that in control group. Also, the NAC injections led to elevate VAP in G3 followed by G2 to be higher than that in G1. This indicates that, sperm in treated groups can travel faster along their average path, as described by Solter *et al*. (2006). Same trend towards increasing was recorded for VCL which was higher in G3 than G1 by 11% and in G2 than G1 by 10 %. Katebi *et al*. (2005) described VCL parameters as an index of swimming speed and in other words, the total distance between adjacement point divided by time elapsed. Larsen *et al*. (2000) stated that, when VCL parameter is > 25 μm/s could be used as predictor for enhancement of natural conception. It was expected that the distance between the first and final tracked point (VSL) also increased in treated groups than the untreated one. The parameters of VSL and VAP could be a bio-indicator of sperm fertilizing capacity as clarified by Solter *et al*. (2006). Meanwhile, those parameters of STR, LIN, WOB were slight higher in G1 than that in G2 and G3. Taken together, the decrease in STR and LIN parameters in treated groups means, the sperm of treated groups follow as less linear (more curved) than those of the control one (Solter *et al*. (2006). The ALH (measuring the ability of sperm to penetrate cervical mucus and fuse with oocyte, Solter *et al*., 2006) was higher in G2 then G3 and both were higher than its level in G1. However, G3 and G1 were almost have similar BCF, the fertility predictive value as defined by Larsen *et al*. (2000), and were higher than that in G2.

Briefly, the present data elucidated that NAC has ability in enhancing semen volume, sperm concentration, plasma membrane intactness, sperm motility parameters (distance and velocity parameters) with increasing sperm ability and fusing with oocyte. All these improvements could be related to NAC effect on enhancing redox system via increasing TAC, CAT and reducing MDA and H_2O_2 in blood as recorded and discussed in aforementioned tabulated data of this study. This is totally agree with Gallo *et al*. (2018),who stated that, NAC ability in reducing OS is mediated via protecting mitochondrial membranes from decay and reducing level of OS. Same accordance is with Jannatifar *et al*. (2019) who recorded improvements in semen volume (by 11%), total motility (by 12.8%) and sperm concentration (by 9.7%), and they attributed these enhancements to ability of NAC modulation of mitochondrial activity.

Many studies examined NAC on semen quality in different species, but as far as we know the present study may be the first one to test impact of NAC on rabbit semen characteristics. For example, Safarinejad and Safarinejad (2009) treated 468

infertile men with 600 mg NAC/day for 26 weeks and compared its effect with selenium. They found that, NAC treatment caused an elevation in sperm count, motility and no abnormalities in sperm morphology were observed. Avdatek *et al*. (2018) administered rats 160 mg NAC / Kg BW daily for 8 weeks. They found that, NAC increases sperm motility and plasma membrane integrity. They attributed their results to ability of NAC in quenching OS and reducing DNA damage that resulted from OS. Furthermore, Rafiee and Tabei (2021) tested NAC impact on men fertility that suffered from COVID-19 by giving them oral administration 600 mg NAC/ day for three months. Those men were suffered from a reduction in sperm volume, concentration and motility and abnormalities in sperm morphology. They recorded a noticeable enhancement in sperm concentration and volume and they confirmed that, NAC has the powerful to enhance and sustain the redox system to reduce ROS levels that impairs sperm functions and morphology. In buffalo, El-Nagar *et al*. (2021) confirmed our finding in the present study. They mentioned that, NAC has a protective effect through in reducing the harmful damage that caused by OS, by elevating TAC and reducing lipid peroxidation. They observed improvements in semen quality due to NAC administration. These improvements included; ejaculate volume, sperm concentration, motility and morphology. Additionally, Partyka *et al.* (2015) found after 24 h of chicken semen storage at 5°C with 15 mM of NAC which increased ($P< 0.05$) the percentage of motile, progressive motile and rapid spermatozoa and also increased the VAP, VSL, VCL, STR and LIN values. It was found that, administration of NAC increased the ALH value. It is well known that NAC, a derivative of amino acid L-cysteine, is mainly used as an antioxidant in semen extenders in chicken (Partyka *et al*., 2015) during liquid storage. Comhaire *et al*. (2000) found that NAC increased sperm concentration and acrosome reaction while reducing ROS and oxidation of sperm DNA in men.

Table 5. Semen characteristics as affected by N-acetylcysteine (NAC) injections during spermatogenesis of NZW rabbit males

Parameters	G1	G2	G3
Volume	0.71 ± 0.08	0.62 ± 0.07	0.76± 0.09
Concentration (10^6/ml)	380.5 ± 51.5	354.4 ±51.5	415.0 ± 51.5
Plasma membrane Intactness %	84.0 ± 1.6	84.5± 1.6	85.8 ± 1.7
Dis Intact %	15.9 ±1.6	15.4 ± 1.6	14.1 ± 1.7
Acrosomal integrity %	87.3 ± 0.6	88.0 ± 0.5	88.7 ± 0.7
Partial damage %	9.0 ± 0.5	8.8± 0.5	8.1 ± 0.6
Complete damage %	3.5 ± 0.2	3.0 ± 0.2	3.4 ± 0.3

No significance differences were recorded among the three groups

Table 6. Sperm motility traits as affected by N-acetylcysteine (NAC) injections during spermatogenesis of NZW rabbit males

Parameters	Control (n = 196)	50 mg (n = 198)	100 mg (n = 199)
Total motility (%)	87.5^{b} ± 0.4	89.8^{a} ± 0.4	90.8^{a} ± 0.4
Progressive motility (%)	75.8^{c} ± 0.5	79.7^{b} ± 0.5	82.5^{a} ± 0.5
DAP (µm)	29.7^{c} ± 0.3	31.0^{b} ± 0.27	32.3^{a} ± 0.3
DCL (µm)	57.2^{b} ± 0.7	62.0^{a} ± 0.7	63.2^{a} ± 0.7
DSL (µm)	23.0^{b} ± 0.3	23.0^{b} ± 0.3	25.0^{a} ± 0.3
VAP (µm/s)	67.3^{c} ± 0.6	71.4^{b} ± 0.6	73.4^{a} ± 0.6
VCL (µm/s)	129.1^{b} ± 1.4	142.2^{a} ± 1.4	143.3^{a} ± 1.4
VSL (µm/s)	52.3^{b} ± 0.7	53.2^{a} ± 0.7	56.9^{a} ± 0.7
STR (%)	77.1^{a} ± 0.5	73.9^{b} ± 0.5	76.7^{a} ± 0.5
LIN (%)	40.8^{a} ± 0.5	37.3^{b} ± 0.5	39.8^{a} ± 0.5
WOB	0.52^{a} ± 0.0	0.50^{b} ± 0.0	0.51^{a} ± 0.0
ALH (µm)	4.9^{c} ± 0.1	5.7^{a} ± 0.1	5.3^{b} ± 0.1
BCF (H_z)	31.3^{a} ± 0.3	29.4^{b} ± 0.3	31.4^{a} ± 0.3

DAP: Distance Average Path (microns); DCL: Distance Curved Line (microns); DSL: Distance Straight Line (microns); VAP: Velocity Average Path (microns/sec); VCL: Velocity Curved Line (microns/sec); VSL: Velocity Straight Line (microns/sec); STR: Straightness (VSL/VAP); LIN: Linearity (VSL/VCL); WOB: Wobble (VAP/VCL); ALH Amplitude of Lateral Head Displacement (microns); BCF: Beat Cross Frequency (H_2), ^{a,b} Means having different superscripts within the same row differ significantly ($P<0.05$)

CONCLUSION

NAC is non-enzymatic antioxidant, it is highly recommended to be used at dose of 100mg NAC / kg BW. That achieved important role in supporting antioxidant system (TAC, MDA, CAT, and H_2O_2, as found in the current study). With no side effects on both liver and kidney functions, which confirm its safety use. Moreover, its important effect on semen

characteristics, such as, the recorded enhancement in sperm volume, concentration, and motility parameters.

REFERENCES

Abdelnour, S., I. El-Ratel, I.P. Soliman, A. A. El-Raghi and S.F. Fouda, 2021. Effects of dietary thyme essential oil on blood haematobiochemical, redox status, immunological and reproductive variables of rabbit does exposed to high environmental temperature. Italian J. Anim. Sci., 21: 51–61. doi.org/10.1080/ 1828051X. 2021.2006807

Ashour, G., S.M. Abdel-Rahman, W.A. Morsy, N. M. Abdel- Azeem and S. A. Barakat, 2018. Physiological, reproductive and productive performance of rabbit does as influenced by N-acetylcysteine administration. Egypt. J. Rabbit Sci., 28: 63-92.

Atef, H.A., M. K. Mansour,M. I. Essam, A. S. Darwish, N.M. Al- Kalamawy, M. A. Ali and M.R. Flourage, 2016. Aflatoxicosis in rabbits with particular reference to its control by N-acetylcysteine and probiotics. Inte. J. Cur. Res., 08: 22548-22560.

Avdatek, F. T., H. D. Ruhi, T.Hüseyin and. B. Yavuz, 2018. Protective Effect of N-Acetylcysteine on Testicular Oxidative Damage, Spermatological Parameters and DNA Damage in Glyphosate-Based Herbicide-Exposed Rats. Vet. J., 11: 292-300.

Çam, Y., G. Eraslan, A. Atasever, M. Erin, B.C.Liman and N. Şeybek, 2008. Efficacy of N-Acetylcysteine on aflatoxicosis in rabbits. Polish J. Environ. Stud., 17: 189-197.

Chaiyasit, W., R.J. Elias, D.J. McClements and E.A. Decker, 2007. Role of physical structures in bulk oils on lipid oxidation; Review. Food Sci. Nutr., 47: 299-317. DOI: 10.1080/10408390600754 24

Comhaire, F., A. Christophe, A. Zalata, W. Dhooge, A. Mahmoud and C. Depuydt, 2000. The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA). 63(3):159–165.

Ducci, M., A. Gazzano, V. Chiara, V.Cela, G.A. Paolo, M. Farnco and R.G. Andera, 2002. Membrane integrity evaluation in rabbit spermatozoa. Europ. Obst. Gynec. Rep. Bio., 102: 53-56.

Duncan, D. E., 1955. Multiple range and Multiple F test. Biometrics, 11: 1-42.

El-Hammady, H. Y., A. A. Abuoghaba, M.G. Abd El-Fattah and H.A. Abd El-Rahman, 2017. Semen physical characteristics, blood parameters and some physiological estimates of rabbit bucks administered with bee pollen under Upper Egypt climatic conditions. Egypt. J. Rabbit Sci., 27: 43-64.

El-Nagar, H.A., M.E.R. Hammad, A.R. Hemida and A.A. Gabr, 2021. Reproductive performance of Egyptian buffalo bulls administrated with N-acetylcysteine, L-carnitine, or their combination. Egypt. J. Nutr. Feeds, 24: 199-209.

Eraslan, G., C. Yucel, M, Eren, B. Camliman, O. Atalay and N. Seybek, 2005. Aspects of using N-acetyl cysteine in alflatoxicosis and its evaluation regarding some lipid peroxidation in rabbits. Bull. Vet. Inst. Pulway., 49: 243 – 247.

Gallo, A., Y. Menezo, B. Dale, G. Coppola, M. Dattilo and E. Tosti, 2018. Metabolic enhancers supporting 1-carbon cycle affect sperm functionality: an in vitro comparative study. Sci. Rep., 8:11769.

García, B.M., L.G. Fernández, C.O. Ferrusola, C. Salazar-Sandoval, A.M. Rodríguez, H.R. Martinez, J.A. Tapia, D. Morcuende and F.J. Peña, 2011. Membrane lipids of the stallion spermatozoon in relation to sperm quality and susceptibility to lipid peroxidation.Reprod. Domest. Anim., 46, 141–148.

Jannatifar, R., K. Parivarand and N.H. Roodbari, 2019. Effects of N-acetyl-cysteine supplementation on sperm quality, chromatin integrity and level of oxidative stress in infertile men. Reprod. Biol. Endocrinol., 17: 24-30. doi.org/10.1186/s12958-019-0468-9

Jeyendran, R. S., H. H. Van der Ven, M. B. Perez-Pelaez, J. Craboand and L.J.D.Zaneveld, 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil., 70: 219–228.

Katebi, M.,M. Mansoureh, A. Mir Abbas, A. Mohammad, A. S.Farid Abolhassani1 and A. Fugaku, 2005. Changes in motility parameters of mouse spermatozoa in response to different doses of progesterone during course of hyperactivation. Iranian Biomed. J., 9: 73-79.

Kordan, W., D. Hoody, B. Eriksson, L. Fraser, H. Rodriguez- Martinez and J. Strze_zek, 1998. Sperm motility inhibiting factor (SMIF) ± a plasmatic peptide with multifunctional biochemical effects on boar spermatozoa. Reprod. Dom. Anim., 33: 347-354.

Larsen, L., T. Scheike, T. K. Jenesen, J.P. Bonde, E. Ernst, H.H. Niels, Y. Y. Zhou, E.S. Niels and A. Giwercman, 2000. Computer-assisted semen analysis parameters as predictor for fertility for men from the general population. Human Rep., 15: 1562- 1567.

McNitt, G.I., S.D. Lukefahr, P.R. Cheeke and N.M. Patton, 2013. Rabbit Production. 9th Ed., CABI, Nosworthy Way, Oxfordshire OX10 8DE MA 02111, UK, Pp: 327.

Mohammadi-Sardoo, M., M. Ali, N. Mohammad, N. M. Seyed-Noureddin and A. Bagher,2018. Mancozeb induces testicular dysfunction through oxidative stress and apoptosis: Protective role of N-acetylcysteine antioxidant. Toxicol Industr. Health, 34: 798–811.

Özkan, C., A. Kaya and Y. Akgül, 2012. Normal values of haematological and some biochemical parameters in serum and urine of New Zealand White rabbits. World Rabbit Sci., 20: 253 – 259.

Partyka, A., W. Niżański, M. Bratkowska and P. Maślikowski, 2015. Effects of N-acetyl-L-cysteine and catalase on the viability and motility of chicken sperm during liquid storage. Reprod. Biol., 15: 126-129.

Pintus, L., and J. L. Ros-Santaella, 2021. Impact of oxidative stress on male reproduction in domestic and wild animals. Antioxidants, 1154: 1-24. https://doi.org/10.3390/antiox10071154.

Rafiee, B., and S.M.B. Tabei, 2021. The effect of N-acetyl cysteine consumption on men with abnormal sperm parameters due to positive history of COVID-19 in the last three months. Arch. Ital. Urol. Andro., 4:465-467

Safarinejad, M.R., and S. Safarinejad, 2009. Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. J. Urol., 181: 741–751.

San-Miguel, B., M. Alvarez, J. M. Culebras, J. Gonz´alez-Gallego and M. J. Tu˜n´on, 2006. N-acetyl-cysteine protects liver from apoptotic death in an animal model of fulminant hepatic failure. Apoptosis, 11:1945–1957 DOI 10.1007/s10495-006-0090-0

SAS, 1999. SAS Users Guide. Statistical Analysis System Institute, Inc., Cary, NC, USA.

Sloter, E., T.E.Schmid, F.Marchetti, B.Eskenazi, J.Nath and A.J.Wyrobek, 2006. Quantitative effects of male age on sperm motion. Human Reprod., 11: 2868–2875.

Sorensen, A. M., 1979. Breeding soundness evaluation. In: Animal Reproduction, Principles and Practices. (Ed. Sorensen, A. M.). McGraw-Hill Book Company, NewYork, USA. pp. 110–146

Verga, M., 2002. Clinical pathology. In: Rabbit Medicine (1st ed.). Linacre House, Jordan Hill, Oxford England., pp. 140 – 164.

Wang, C., Y. Xia, Y. Zheng, W. Dai, E. Wang, K. Chen, J. Li, S. Li and R. Zhu, 2015. Protective effects of N-acetylcysteine in concanavalin a-induced hepatitis in Mice. J. Med. Inflamm., 2:1-17.

Watson, P.F., 1975. Use of a giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. Vet. Rec., 5: 12-15.

التأثير الوقائي لمركب إن أستيل سستين ضد الإجهاد التأكسدي وعلاقته بخصائص السائل المنوي في الأرانب النيوزيلندي البيضاء

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لتقييم تأثير مركب أن أستيل سستين(NAC) على حالة مضادات الأكسدة وخصائص السائل المنوي في ذكور الأرانب النيوزيلندي البيضاء (NZW) التامة النمو. تم استخدام ١٥ ذكر تتراوح أعمارها بين ٦-٨ أشهر ، ووزنها حوالي ٣.٥ كجم كمتوسط . تم تقسيم الذكور المستخدمة إلى ثلاث مجموعات متساوية ، المجموعةالضابطه (G1) التي تم حقنها تحت الجلد بمحلول ملحي. تم حقن المجموعتين الأخريين (G2) و (G3) تحت الجلد بجرعة ٥٠ و ١٠٠ ملجم NAC / كجم من وزن الجسم ، على التوالي ، واستمر الحقن لمدة سبعة أيام متتالية. بعد ذلك ، تم جمع عينات الدم في اليوم الثامن (بعد نهاية حقن NAC بيوم واحد) وفي اليوم ٤٧ (قبل جمع السائل المنوي بيوم واحد) لإجراء تحاليل الدم لكلا من مضادات الأكسدة ووظائف الكبد والكلى. بلإضافة الي ذلك ، تم تقييم خصائص السائل المنوي في اليوم ٤٨. استمرت التجربة لمدة ٩٦ يومًا بشكل مستمر. أظهرت النتائج التأثير الإيجابي لحقن NAC على حالة مضادات الأكسدة من خلال تأثيره الواضح على مستوى MDA الذي انخفض بشكل مستمر، في G3 ، من ٦.١٠ إلى ٣.٥٨ نانومول / مل ، من بداية التجربة إلى يوم ٤٧. بينما أظهرت مجموعة G1 زيادة مستمرة في مستواه. أيضا، نجح حقن NAC بجرعة ١٠٠ ملجم / كجم من وزن الجسم في رفع نشاط انزيم الكتاليز الذي ارتفع من ٣٢٩.٨ إلى ٣٧٢.٤ وحدة / لتر في اليوم الثامن مقارنة بمستوياته في يوم الصفر وهو اليوم قبل حقن المركب. علاوة على ذلك ، انخفض H_2O_2 بشكل ضئيل واستمر هذا الانخفاض حتى اليوم ٤٧ في G3 (انخفض من ٠.٠٩٣ في اليوم صفر إلى ٠.٠٨٣ مليمول / لتر ، في اليوم ٤٧) في حين كان مستواه شبه ثابت في المجموعة G2بينما كانت وظائف كلا من الكبد والكلية طبيعية كما تأكد من مستويات إنزيمات الكبد (ALT و AST) وكذلك مستويات الكيرياتنين واليوريا بالنسبة لوظائف الكلية في الأرانب المعاملة. ، .تم تسجيل تحسن في خصائص السائل المنوي بسبب حقن NAC (١٠٠ مجم / كجم من وزن الجسم). خاصة تركيز الحيوانات المنوية أعلى بكثير في ال G3 (٤١٥.١٠٦ / مل) من G2 (٣٥٤.٤١٠٦ / مل) و G1 (٣٨٠.٥١٠٦ / مل).
أثبتت الدراسة الحالية أن استخدام NACبجرعة ١٠٠ملجم / كجم من وزن الجسم في ذكور الأرانب يؤثر إيجابًا على حالة مضادات الأكسدة وخصائص السائل المنوي لذكور الأرانب .