Original Research

Journal of Advanced Veterinary Research
(2025) Volume 15, Issue 1, 28-34

Effect of vitamin C and its nanoparticles injection on testicular hemodynamics, testicular volume, testicular echotexture, and circulating testosterone and nitric oxide in pubescent goat bucks

Mayada Essam[1], Mohamed A.I El Sayed[1], Fady S. Youssef[2], Haney Samir[1*]

[1]*Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.*
[2]*Pharmacology Department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.*

ARTICLE INFO

Recieved: 02 October 2024

Accepted: 28 November 2024

*Correspondence:

Corresponding author: Haney Samir
E-mail address: Haneyvet360@cu.edu.eg

Keywords:

Vitamin C, Nanoparticles, Testicular hemodynamics, Nitric oxide, Testosterone, Estradiol, Goats.

ABSTRACT

The current study investigated the effect of vitamin C and its nanoparticles (NPs) on testicular hemodynamics using Doppler ultrasonography, testicular volume (TV), testicular echotexture (PIX), and circulating testosterone (T), nitric oxide (NO), and total antioxidant capacity (TAC) in pubescent bucks under heat stress conditions. Fifteen Baladi goat bucks were split into three groups (5 in each): the control group received subcutaneous (S/C) injections of 1 ml of corn oil, the vitamin C group received S/C injections of 1 ml of traditional vitamin C (5mg/kg body weight), and the vitamin C NPs group was administered by S/C injections of 1ml of vitamin C NPs (1.25 mg/kg body weight). All groups had been injected twice a week in 3-4 days intervals for 4 consecutive weeks. Testicular hemodynamics assessment was done on day zero, 4, 8, 12, 16, 20, 24, 28, and 32. Estimating the TV and PIX was performed by B-mode ultrasonography and computer-assisted image analysis software. Concentrations of T, NO, and TAC were measured using commercial kits. Results revealed significant decreases ($P < 0.05$) in Doppler indices values (resistive index and pulsatility index) in the vitamin C NPs group followed by the vitamin C group compared to the control group. There were significant increases ($P < 0.05$) in the TV, PIX, and NO, TAC concentrations in the vitamin C NPs group and vitamin C group compared to the control group. There were no significant differences ($P > 0.05$) in the concentrations of T between all groups during the studied time points. In conclusion, vitamin C NPs improve testicular blood flow and increase NO concentrations and TV than traditional vitamin C. Nano-formulations of vitamin C showed higher antioxidant activity compared to traditional vitamin C as it improves its stability and bioavailability. So, using nanoparticles of vitamin C could be recommended for improving the reproductive performance of pubescent bucks under heat-stress conditions.

Introduction

Goats in Egypt are one of the important livestock because they are considered an important source of meat and milk production (Marai *et al*., 2002; Galal *et al*., 2005). In the last decade, there has been a decline in the productivity of goats. Therefore, different strategies that aim to raise male and female reproductive capacities are of great value. In small ruminant herds, it is essential to choose males with high reproductive performance potential (Saaed and Zaid, 2018; Camela *et al*., 2019). Male spermatogenesis and normal testicular function are temperature-sensitive because heat stress damages all of the major testicular cells, lowering the quality and quantity of sperm. This explains why the testes in the majority of mammals are situated outside the body in the scrotum to maintain the intratesticular temperature at approximately 32°C, which is somewhat lower than the body's core temperature, for proper spermatogenesis (Paul *et al*., 2008; Hansen, 2009; Shahat *et al*., 2020).

The increased temperatures and relative humidity in summer months could be contributing to the extra decline in bucks' reproductive performance during the non-breeding season (Lu, 1989; Samir *et al*., 2018). The decline of reproductive performance in goat bucks may be caused by oxidative stress conditions brought on by summer heat stress (Lu, 1989). Heat stress (HS) generates an excess of free radicals (hydroxyl radical, superoxide anion radicals, singlet oxygen, and hydrogen peroxide), which are constantly generated during aerobic metabolism (Bernabucci *et al*., 2002) and can damage healthy cells if they remain inside. As a result, HS reduces reproductive performance, hinders spermatogenesis (Kay *et al*., 1992), and has an impact on testicular hemodynamics, semen quality, and the fertilizing potential of goat bucks (El-Sherbiny *et al*., 2022a). Because of the detrimental effects of oxidative stress on testosterone synthesis, testicular blood flow, sperm quality, and fertility, (Bansal and Bilaspuri, 2011; El Tohamy *et al*., 2012; Hedia *et al*., 2020), numerous studies were conducted to mitigate the adverse effects of HS on testicular functioning using hormones (gonadotrophin) in bucks (Samir *et al*., 2015; Abbas *et al*., 2021) and antioxidants (Vitamin C) in goats and (L-carnitine) in rams (Minka and Ayo, 2010; 2012; EL-Sherbiny *et al*., 2022b).

Vitamin C is a potent antioxidant and plays a crucial role in immune function regulation and protects from oxidative damage at many cellular and sub-cellular levels by scavenging free radicals and preventing membrane phospholipids from peroxidation (Englard and Seifter, 1986; Ranjan *et al*., 2012) and as reported in goats, its oral supplementation reduces the stress-induced hemolysis of erythrocytes following road transportation for 12 hours during hot-dry season (Minka and Ayo, 2010). Along with all of these functions, it is essential for several mammalian species' reproduction. Earlier investigations have demonstrated the advantages of ascorbic acid subcutaneous injections to sub-fertile bulls (Phillips *et al*., 1940), cows (Phillips *et al*., 1941), and stallions (Ralston *et al*., 1988). Additionally, it has been reported that vitamin C was utilized therapeutically to alleviate infertility in adult ruminants, enhancing the performance of bulls with poor breeding records, and in cows, boosting the rate of conception, and lowering the retained placenta cases (Daykin, 1960). Vitamin C supplementation lowers the incidence of secondary sperm abnormalities via lowering the formation of free radicals (Sikka, 1996), involved in sex steroids synthesis including testosterone; hydroxylation of steroids is particularly dependent on vitamin C (Luck *et al*., 1995; Weber *et al*., 1996) and can enhance sperm viability and normal morphology in goats (Fazeli *et al*., 2010). It raises the catalase concentration and enhances spermatogenesis, sperm concentration, motility, and viability (Raeeszadeh *et al*., 2021). Ascorbic acid may play an essential role in fertility and be regarded as an essential element in the reproductive process (Luck *et al*., 1995), as it is essential in protecting spermatozoa from oxidative damage in vivo,

ISSN: 2090-6277/2090-6269/

with approximately seven times higher concentrations of ascorbic acid in seminal fluid in comparison with blood plasma concentration (Fraga *et al*., 1991). Its deficiency causes a reduction in sperm counting and increases in morphological abnormalities (Ebesunun *et al*., 2004), along with increased levels of oxidative sperm DNA damage (Fraga *et al*., 1991).

The poor stability of vitamin C impedes its therapeutic use. Vitamin C is unstable due to a variety of physical and chemical factors, including temperature, light, metal ions, enzymatic oxidation, ambient oxygen, and alkaline pH, which causes it to break down rapidly into less active species (Herbig and Renard, 2017). The development of efficient delivery systems that can transport vitamin C to the bloodstream is essential for the preservation of vitamin C by protecting it from environmental factors with the possibility of controlled releasing capacities (Comunian *et al*., 2022). Incorporating vitamin C into nano-carriers was found to improve its solubility, stability, and bioavailability as well as its ability to cross barriers (Alishahi *et al*., 2011; Mikirova *et al*., 2019; Gopi and Balakrishnan, 2021; Subroto *et al*., 2021). Male infertility has been reversed by vitamin C in nanoparticles, according to Zhao *et al*. (2012). When compared to traditional vitamin C, vitamin C nanoformulations exhibit higher antioxidant activity (Chae and Park, 2007; Parhizkar *et al*., 2018). For example, it was revealed that with gingival inflammation cases, an emulsion of nano-vitamin C showed antioxidant capabilities (Chae and Park, 2007).

Baladi goats in subtropical climates are more susceptible to HS than crossbreeds (Teama and El-Tarabany, 2016; Al-Dawood, 2017; El-Tarabany *et al*., 2017). Overall, we speculated that administration of vitamin C, in its nanoparticle forms, could be beneficial to combat the adverse effects of HS conditions on testicular function in male goats by increasing testicular blood flow, testosterone (T) levels, and testicular volume. Therefore, the objective of this study was to investigate and compare the effect of vitamin C and vitamin C nanoparticles (Vitamin C NPs) on testicular hemodynamics, testicular volume, testicular echotexture, and circulating testosterone, nitric oxide, and total antioxidant capacity in pubescent bucks under HS conditions (during summer months).

Materials and methods

The study was conducted on male Baladi goats. Baladi goats are the most common breed raised in Egypt's Nile Delta which is crucial to Egypt's production of milk and meat. They are a non-seasonal breed and reach puberty at the age of 4 to 6 months (Galal *et al*., 2005).

All methods in this study were performed following the rules and regulations of the Ethics Committee for Animal Use at the Faculty of Veterinary Medicine, Cairo University, Egypt (approval number: Vet CU 25122023889).

Materials

Vitamin C (L-Ascorbic Acid), Surfactant (non-ionic) (Tween 80 and Tween 20), and deionized water were obtained from Sigma-Aldrich Company, and corn oil was obtained from Oleum Company Egypt.

Animals and management

This study was performed at the Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University. Fifteen Baladi bucks (Capra hircus) weighing 15 - 20 kg, and 5-6 months- old, were included in this study during September 2023. Based on data from the Egypt Meteorological Agency, the average temperature (T), relative humidity (RH), and temperature humidity index (THI) were 35.88±1.2°C, 49.38±3.45%, and 85.6±0.96 for the duration of the experiment. To determine if bucks in this experiment were heat-stressed, the following previously published formula (Kendall and Webster, 2009; El-Tarabany *et al*., 2017) was utilized: THI = (1.8 × T + 32) − [(0.55 − 0.0055 × RH) × (1.8 × T − 26)]. The three stress levels of the buck were ascertained by this: not stressed (THI < 70), moderately stressed (THI = 70 – 80), and severely stressed (THI > 80). The bucks that were examined (THI = 85.6±0.96) were therefore considered to be under severe HS. Bucks were housed indoors and fed according to NRC guidelines with clean water availability ad libitum. Before starting the study, bucks were exposed to routine clinical examinations which included full ultrasonographic scans of the reproductive tract to exclude the existence of any abnormalities. Bucks were regularly subjected to vaccination and deworming against endemic diseases in Egypt.

Preparation of nanoparticles of vitamin C

Method of preparation

After 8 mg of vitamin C was mixed with 25 ml of tween 20 and 25 ml of tween 80 using a probe homogenizer (LK Lab, Korea). Fifty ml of deionized water was gradually added to the mixed oil phase (corn oil). The procedure for the preparation of nanoemulsion was followed by Silva *et al.* (2011).

Isolation and drying

To sediment the formed vitamin C nano-emulsion, the prepared vitamin C nano-emulsion was centrifuged at 20,000 rpm. After 3 to 4 times of washing, the nanoparticles were vacuum-dried overnight at 40°C.

Method of characterization

We have examined the physical and chemical characteristics of the vitamin C nano-emulsion to evaluate its biological capabilities. The shape and surface topography of the vitamin C nanoemulsion were determined using microscopic analysis. TEM (Transmission Electron Microscopy) model of Jeol, JEM-2100 high-resolution, Japan, and AFM (Atomic force microscopy), Agilent, USA were used to index, identify, and characterize the specimen. The ability of particles to disperse in fluids was determined by determining the zeta potential and size using DLS (dynamic light scattering) (Malvern, UK).

Antioxidant activity of Vitamin C nano-emulsion in vitro

Baliyan *et al*. (2022) used the stable radical DPPH to evaluate the free radical scavenging abilities of Vitamin C nano-emulsion compared to regular vitamin C. One mL of Vitamin C nano-emulsion at different concentrations (1000, 500, 250, 125, 62.5, 31.2, 15.6 µg/mL) with 1.0 mL of DPPH (1.0 M in methanol) was centrifuged, thereafter 30 minutes incubation at room temperature. A UV-VIS spectrophotometer (Systronics, AU-2701) was used to measure the absorbance at 517 nm. We also tested the sample with DPPH and chemicals that were not in the sample as a control. We used a methanol blank solution. The following formula was used to measure free radical scavenging activity:

Percentage of scavenging = (Pc − Ps)/Pc × 100.

Where Pc= absorbance of vitamin C and Ps = Vitamin C nano-emulsion.

Experimental design

All experimental procedures are illustrated in Figure 1. Bucks were split into 3 groups (5 in each group): (1) the control group, which received subcutaneous (S/C) injections of 1 ml of corn oil, (2) the vitamin C group, which received S/C injections of 1 ml of traditional vitamin C (5mg/kg body weight). The dose was chosen based on a previous study in goats (Daykin, 1960), (3) the vitamin C NPs group, which received S/C injections of 1ml of vitamin C NPs (1.25 mg/kg body weight) (Shadman *et al*., 2022). All groups were injected twice a week in 3-4 days intervals for 4 weeks based on previous literature (Daykin, 1960). Bucks were examined for hemodynamics parameters [resistive index (RI) and pulsatility index (PI)] in

the supra testicular artery (STA), testicular echotexture, and testicular volume at 3-4 days intervals post-injection. Blood samples were collected at the same time as ultrasonographic scanning, to determine the variations in the circulating testosterone (T), nitric oxide metabolites (NO), and total antioxidant capacity (TAC) in the sera.

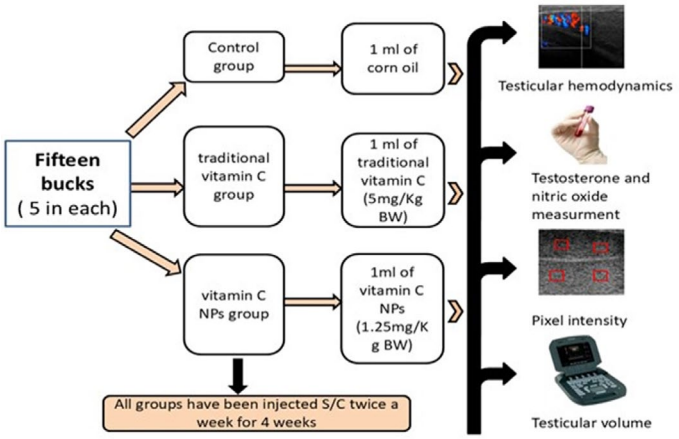

Fig. 1. Schematic diagram of the experimental design.

Blood sampling

Blood samples (3 ml) were collected from the jugular vein of each buck at each ultrasound examination time in plain tubes (Vacutest; Shandong Yaohua, China). Three ml of blood was collected and then centrifuged at 3000 rpm for 15 minutes. The serum was separated and stored at −20°C until further analysis.

Hormonal and biochemical analysis

Testosterone concentration (ng/mL) was measured in sera samples using a commercial ELISA kit (Diasino Laboratories Co., Ltd, China) with an assay sensitivity of 0.055 ng/ml and the intra-and inter-assay coefficients of variation were 3.3 and 4.8%, respectively.

Concentrations of NO metabolites and TAC were measured calorimetrically by a spectrophotometer, to be set, at 540 and 505 nm wavelength respectively using commercial kits (Nitric oxide kit and total antioxidant capacity, Bio-diagnostic Co., Dokki, Giza, Egypt) following the instructions of the manufacturer.

Ultrasonographic examinations

All ultrasonographic scans were performed by the same operator using the ultrasound scanner (EXAGO, Echo Control Medical, IMV, France) supplied with a 5-7.5 MHz linear transducer. Before starting the ultrasonographic examinations, each animal was restrained without sedation then recumbent on the right side, and the hair on the scrotum till the spermatic cord was shaved carefully without injuries. A sufficient amount of ultrasonic gel was applied to the probe eliminating air spaces to facilitate the ultrasonographic assessment.

Testicular volume and echotexture assessment using B-mode ultrasonography

For determining the testicular volume (TV), each testis was scanned gently by B-mode ultrasonography without pressure until the appearance of mediastinum testis. Images of each testis, longitudinal and cross-section, were recorded. The testicular length (L), width (W), and height (H) were measured using electronic calipers from the frozen B-mode images. These measurements were used in the following formula for calculating the testicular volume:

TV (ml) = L* W* H* 0.61 (Montes-Garrido *et al*., 2023).

For assessing the echotexture of the testicular parenchyma (pixel intensity {PIX}), each testis was imaged in longitudinal and cross sections for further analyses using computer-assisted image analysis software (Adobe Photoshop CC program) as previously described (Ahmadi *et al*., 2012; Brito *et al*., 2012; Pozor *et al*., 2017). The transducer was put parallel to the longitudinal axis of the testis for the longitudinal sections, and for the cross-sections, the transducer was perpendicular to the longitudinal axis of the testis (Samir *et al*., 2018). On saved images of homogeneous testicular parenchyma, four rectangular-shaped areas above and below the mediastinum were chosen and selected for each testis separately excluding any artifact in the images. The results of the right and left testes were averaged for analysis.

Testicular hemodynamics evaluation

To examine the hemodynamic changes within the STA, the probe was placed vertically on the proximal pole of the testis and then moved upward till the appearance of the vascular network and identified the supratesticular artery in the pampiniform plexus by using B-mode ultrasonography. Following that, the color and pulsed Doppler modes were activated to visualize the blood flow waveforms within the examined segment of the STA. However, to differentiate the STA from the venous network, the artery had a waveform on the spectral graph following the arterial pulse in each cardiac cycle (systole and diastole), while the flow in the veins was relatively constant without a distinct pulse (Samir *et al*., 2015, 2018). After the spectral pattern of the STA appearance (Fig. 2), three to -five successive waves with complete systolic and diastolic cardiac cycles were measured for assessment of Doppler indices as RI, PI, and systolic/diastolic ratio (S/D ratio) (Gumbsch *et al*., 2002). All spectral-Doppler settings including focus, brightness, contrast, and gain were kept constant for all examinations. The angle between the long axis of the examined vessel and the Doppler beam was < 60 degrees in the blood flow direction (Fig. 2).

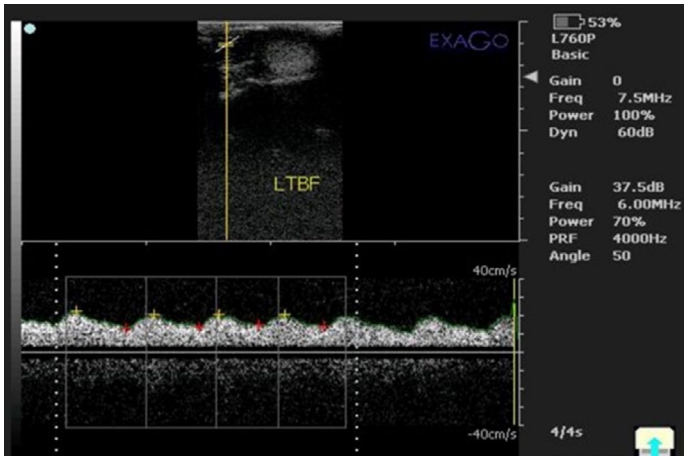

Fig. 2. The spectral pattern of the STA using color-pulsed Doppler ultrasonography.

Statistical analysis

GraphPad Prism 5 software was used in all statistical analyses. The data normality was examined by the Kolmogorov-Smirnov test. In this study, all data were normally distributed and expressed as the mean±-standard error of the mean (SEM). The differences between control, vitamin C, and vitamin C NPs groups, in terms of testicular volume, testicular echotexture (PIX), testicular blood flow (RI, PI, and S/D ratio), T, NO concentrations, and total antioxidant capacity throughout the studied time points (twice/week for 4 weeks; time effect) and combined treatment time effect was analyzed by two-way ANOVA test. A probability value of at least < 0.05 was considered significant. The differences between the left and right testis were non-significant; therefore, data from both testes was pooled and expressed per buck.

Results

Characterization of Vitamin C Nano-emulsion

The results of characterization techniques have been illustrated in Figure 3. A TEM image of the Vitamin C nano-emulsion, as shown in Fig. 3A, revealed that the particles were spherical to subspherical with a maximum thickness of 25-50 nm. Additionally, it was found that Vitamin C Nanoemulsion does not tend to agglomerate in certain regions. Vitamin C Nano-emulsion is spherical without any agglomeration with a homogeneous well-dispersed matrix as demonstrated by TEM and AFM images (Fig. 3A, 3B). The results of zeta size showed that the average particle size of synthesized Vitamin C Nanoemulsion is 33.8±0.41 nm as shown in Fig. 3C. Based on the results, it can be concluded that the high zeta potential of the synthesized Nanoemulsion as shown in Fig. 3D, directly affects the colloidal stability in water, since it is obtained from the high bioactivity of the Vitamin C Nanoemulsion.

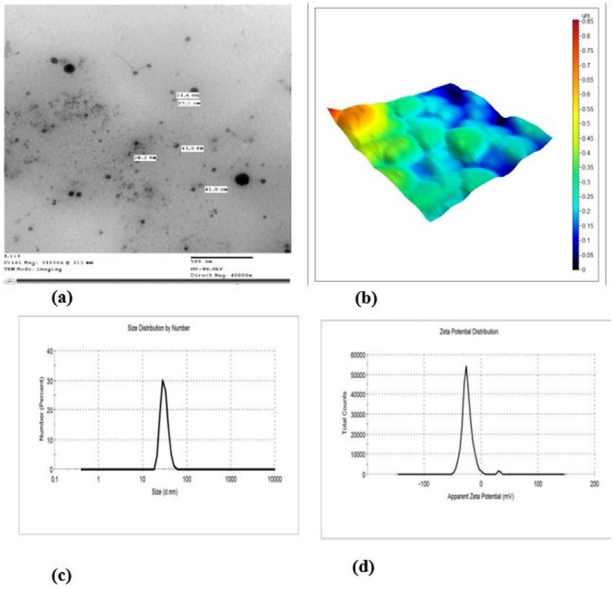

Fig. 3. illustrates the results of characterization techniques of vitamin C nano-emulsion: A) Imaging the vitamin C nano-emulsion by transmission electron microscopy (TEM) showed that the particles were spherical to subspherical without any agglomeration. B) imaging the vitamin C nano-emulsion by atomic force microscopy (AFM) revealed that all particles are homogeneous with a well-dispersed matrix. C) The ability of particles to disperse in fluids was determined using dynamic light scattering (DLS) that showed the average particle size (zeta size) of synthesized vitamin C Nanoemulsion is 33.8±0.41 nm. D) panel showed the high zeta potential of the synthesized nano-emulsion.

Antioxidant activity of Vitamin C nano-emulsion in Vitro

At the various tested concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56 µg/mL) represented as 1, 2, 3, 4, 5, 6, 7 in Fig. 4, Vitamin C nano-emulsion showed antioxidant activity. The percentage of antioxidant activity increased in a dose-dependent manner that was comparable to that of standard ascorbic acid.

Testicular hemodynamics

The effect of vitamin C NPs and vitamin C administration on testicular hemodynamics in pubescent bucks is shown in Figure 5. There were treatment and time effects ($P < 0.05$) on the values of RI, PI, and S/D ratio. The interaction (treatment x time) factor showed non-significant effects on all studied Doppler parameters. There was a significant decrease in Doppler indices in the vitamin C NPs group followed by the vitamin C group compared to the control group. Significant decreases in the RI, PI, and S/D ratio values were found nearly at all the studied time points in the vitamin C NPs group. In the vitamin C group, there were significant decreases in the RI value on days 8, 12, and 28 compared to the control group. However, the PI and S/D ratio attained lower values from day 2 till the end of the study compared to the control group.

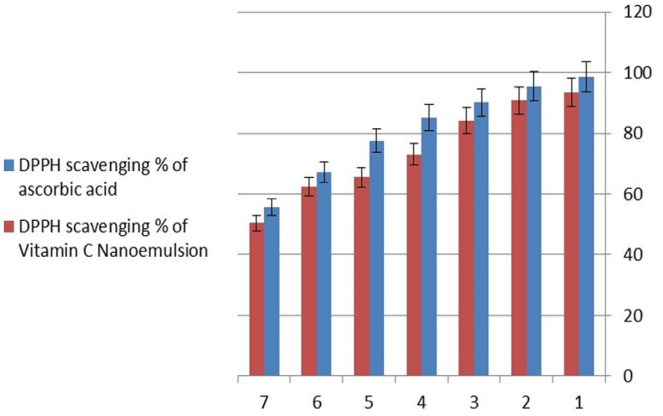

Fig. 4. demonstrates the % of free radical scavenging activity of standard ascorbic acid and vitamin C nano-emulsion.

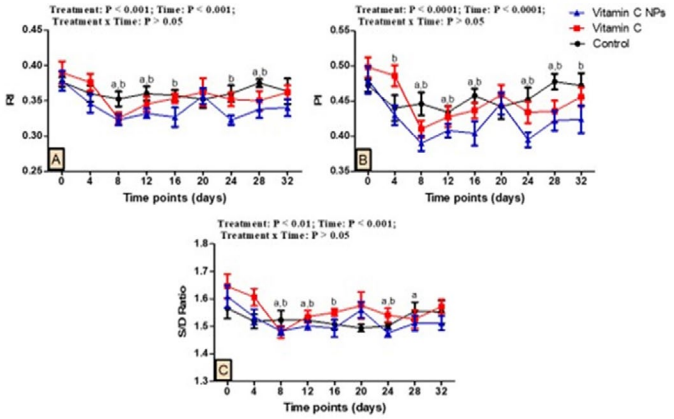

Fig. 5. Changes in the parameters of testicular hemodynamics [RI (A), PI (B), and S/D ratio(C)] at the level of supratesticular artery as measured by pulsed-wave Doppler ultrasonography in pubescent goat bucks in the vitamin C nanoparticles (NPs) group, vitamin C group, and the control group (n = 5, each) during different time points (days). Values are means±SEM. Values with superscripts A represent a significant difference ($P < 0.05$) between vitamin C and control groups during the study, while those with superscripts B represent a significant difference ($P < 0.05$) between vitamin C NPs and control groups during the study. Values with superscripts C represent a significant difference ($P < 0.05$) between vitamin C NPs and vitamin C groups at indicated time points. Values with superscripts a, and b represent significant differences ($P < 0.05$) between day 0 and the indicated point within the vitamin C and vitamin C NPs group, respectively.

Testicular volume and echotexture

The effect of vitamin C NPs and vitamin C administration on testicular volume and echotexture (PIX) is shown in Figure 6. Generally, there were treatment, time, and their interaction effects ($P < 0.0001$) on the values of TV. Also, there were treatment, time, and their interaction effects ($P < 0.0001$, $P < 0.0001$, and $P < 0.01$, respectively) on the values of PIX. Bucks in the vitamin C NPs and vitamin C groups had significantly high values of the TV ($P < 0.001$) at all studied time points compared to the control group. The TV value, especially on day 20, was significantly higher in the vitamin C group (86.3±1.09) compared with the vitamin C NPs group (77.2±1.04, $P < 0.01$). There was a significant increase ($P < 0.001$) in PIX values at all studied time points in the vitamin C NPs and vitamin C groups compared to the control group. The PIX value, especially on day 4, was significantly higher in the vitamin C NPs group (105.7±2.2) compared with the vitamin C group (97.6±2.04, $P < 0.05$).

Hormonal and biochemical analysis

The effect of vitamin C NPs and vitamin C administration on testosterone, nitric oxide metabolites, and total antioxidant capacity is shown in Figure 7. In general, there were time and interaction (treatment x time) effects on the T concentrations ($P < 0.0001$, $P < 0.001$). Significant in-

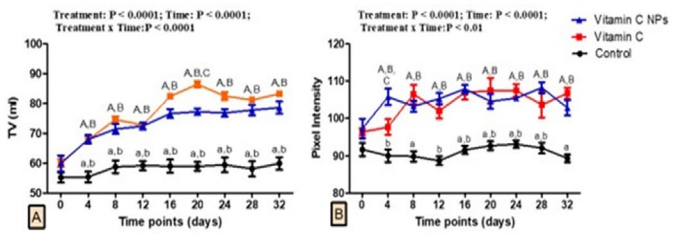

Fig. 6. Changes in the testicular volume (A) as measured by B-mode ultrasonography and testicular echotexture (B) [pixel intensity; PIX] as measured by computer analysis software in pubescent goat bucks in the vitamin C nanoparticles (NPs) group, vitamin C group, and the control group (n = 5, each) during different time points (days). Values are means±SEM. Values with superscripts A represent a significant difference (P < 0.05) between vitamin C and control groups during the study, while those with superscripts B represent a significant difference (P < 0.05) between vitamin C NPs and control groups during the study. Values with superscripts C represent a significant difference (P < 0.05) between vitamin C NPs and vitamin C groups at indicated time points. Values with superscripts a, and b represent significant differences (P < 0.05) between day 0 and the indicated point within the vitamin C and vitamin C NPs group, respectively.

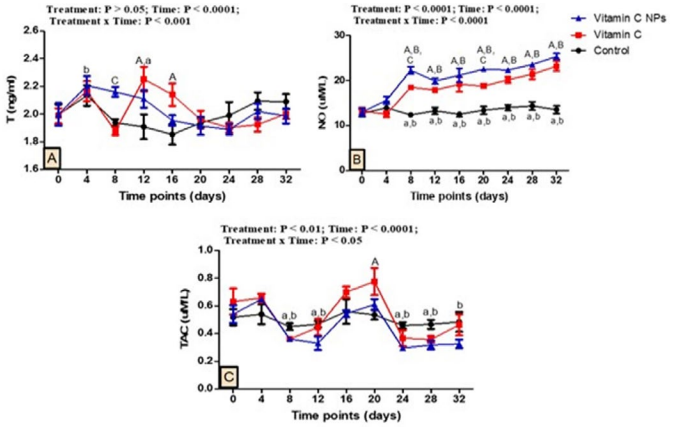

Fig. 7. Changes in testosterone (T; ng/mL) (A), nitric oxide concentrations (NO; µmol/L) (B), and total antioxidant capacity (TAC; mM/L) (C) in pubescent goat bucks in the vitamin C nanoparticles (NPs) group, vitamin C group, and the control group (n = 5, each) during different time points (days). Values are means±SEM. Values with superscripts A represent a significant difference (P < 0.05) between vitamin C and control groups during the study, while those with superscripts B represent a significant difference (P < 0.05) between vitamin C NPs and control groups during the study. Values with superscripts C represent a significant difference (P < 0.05) between vitamin C NPs and vitamin C groups at indicated time points. Values with superscripts a, and b represent significant differences (P < 0.05) between day 0 and the indicated point within the vitamin C and vitamin C NPs group, respectively.

creases (P < 0.05) in T concentrations were found in the vitamin C group on days 12, and 16 (2.254±0.08 ng/ml and 2.14±0.08 ng/ml, respectively) compared with the control group at the same time points (1.9±0.08 ng/ml and 1.8±0.07 ng/ml, respectively). Pubescent bucks in the vitamin C NPs group attained a significant increase (P < 0.05) in T concentration on day 8 (2.16±0.03 ng/ml) compared with that in the vitamin C group (1.87±0.02 ng/ml). Regarding the time factor, the highest concentration of T (2.20±0.06 ng/ml) was recorded on day 4 in the vitamin C NPs group, while it reached the highest concentration (2.25±0.08 ng/ml) on day 12 in the vitamin C one.

There were treatment, time, and their interaction effects (P < 0.0001) on the NO concentrations. There were significant increases (P < 0.05) in the concentrations of NO at all studied time points in the vitamin C NPs group followed by the vitamin C group compared to the control group. Concentrations of the NO were significantly high in the vitamin C NPs group especially on days 8, and 20 (22.2±0.8 and 22.5±0.5 µmol/L, respectively) compared with the vitamin C group at these time points (18.4±0.5 and 18.7±0.5 µmol/L, respectively).

There were treatment, time, and their interaction effects (P < 0.01, P < 0.0001, and P < 0.05, respectively) on total antioxidant capacity values. There was a significant increase (P < 0.05) in TAC value in the vitamin C group on day 20 (0.77±0.09 mM/L) compared to the control group (0.53±0.03 mM/L) at the same time point.

Discussion

Vitamin C is a strong antioxidant and gives protection against oxidative damage at several cellular levels by acting as a free radical scavenger by inhibiting membrane phospholipids peroxidation (Englard and Seifter, 1986; Ranjan *et al*., 2012). It is regarded as a crucial biochemical in the reproductive process and as potentially having a significant effect on fertility (Luck *et al*., 1995), as it is essential in protecting spermatozoa from oxidative stress in vivo (Fraga *et al*., 1991). Due to the poor stability of vitamin C which impeded its therapeutic uses, the development of efficient delivery systems is necessary. We hypothesize that the administration of vitamin C, in its nanoparticle forms, could be more beneficial than traditional vitamin C to combat the adverse effects of HS conditions on testicular function in male goats by increasing testicular blood flow, testosterone (T) levels, and testicular volume. The findings of the present work supported our hypothesis. The blood flow of the testis is essential to its function as any disruptions to this flow may impair spermatogenesis due to inefficient mitochondrial energy metabolism (Ribeiro *et al*., 2020; Abdelnaby *et al*., 2021). Male reproductive capabilities can be reliably assessed using testicular hemodynamics assessment (Gloria *et al*., 2020; Ribeiro *et al*., 2020). The RI and PI are frequently applied in clinical studies of testicular blood flow in both humans and animals under physiological and pathological conditions (Günzel-Apel *et al*., 2001; Biagiotti *et al*., 2002; Gumbsch *et al*., 2002; Pozor and McDonnell, 2004; Bumin *et al*., 2007; Dudea *et al*., 2010). According to our findings, the Doppler indices (RI and PI) within the STA in the vitamin C NPs group followed by the vitamin C group were significantly lower than the control group. Doppler indices and blood flow were inversely correlated. Decreased blood flow resistance and increased testicular vascular flow are indicated by decreased RI and PI values, which maintain the testis's constant oxygen and nutrient supply (Dickey, 1997; Ginther, 2007). One possible explanation for the increase in testicular blood flow in the NPs group could be attributable to the higher antioxidant activity of vitamin C in its nanoformulations when compared to traditional vitamin C (Chae and Park, 2007; Parhizkar *et al*., 2018). These could improve its solubility, stability, and bioavailability as well as its ability to cross barriers (Alishahi *et al*., 2011; Mikirova *et al*., 2019; Gopi and Balakrishnan, 2021; Subroto *et al*., 2021), and collectively moore protection of testicular blood vessels and reduction of the endothelial oxidative damage to properly function.

Numerous peripheral organs produce nitric oxide, such as the testicular endothelium and seminiferous tubules (Paulis and Simko, 2007). Owing to its potent vasodilatory effect, some studies have suggested that NO is essential for regulating testicular hemodynamics and vascular tone (Lissbrant *et al*., 1997; Paulis and Simko, 2007; Abdelkhalek *et al*., 2022). Our findings revealed an increase in NO concentration in the vitamin C NPs group followed by the vitamin C group than the control group. As vitamin C acts as a free radical scavenger, it protects the testicular endothelium from damage during oxidative stress states and prevents vascular dysfunction. Our finding was consistent with previous studies (Khalili *et al*., 2020; Schwartz-Duval *et al*., 2020). In rats, vitamin C NPs reduced systolic blood pressure in contrast to the free compound, and this action is mediated through nitric oxide production (Khalili *et al*., 2020). According to the preceding data, the decrease in Doppler indices may also be attributed to the rise in NO concentration following the administration of vitamin C and its NPS.

B-mode ultrasonography has been regarded as an accurate and reproducible method for assessing testicular function and pubertal development, as well as TV (Takihara *et al*., 1983; Sakamoto *et al*., 2008). The estimation of testicular volume is crucial for ensuring reproductive capacity, as it is a significant indicator of sperm cell production. This is

because the testis is a highly compact organ (with seminiferous tubules comprising 70-80% of the testicular parenchyma) (Sarlós *et al*., 2013). There was an increase in TV in our study in both treated groups compared to the control one. Increasing TV could be attributable to changes in seminiferous tubules, interstitial cells, and the spermatogenesis process. As previously reported, vitamin C and its nanoparticles enhanced the deposition of seminiferous tubules and boosted the spermatogenesis process (Raeeszadeh *et al*., 2021; Rauf *et al*., 2021). Additionally, increased TV could be due to the decreased RI and PI values of the blood flow within the testicular artery, as there was an inverse correlation between TV and the RI and PI of the STA (Samir *et al*., 2015; Ribeiro *et al*., 2020). Additionally, the low Doppler indices of the testicular artery are consistent with the increase in testicular volume (Strina *et al*., 2016). Decreased RI and PI of the STA may result in increased testicular blood flow and interstitial fluid (IF), and this correlation may explain the TV increases. Our results were contrary to those previously reported (Fazeli *et al*., 2010).

Testicular echogenicity is regarded as a complementary noninvasive method for estimating the testicular function by assessing some parameters such as pixel intensity (Brito *et al*., 2012). In our study, there were significant increases in the PIX in vitamin C NPs and vitamin C groups compared to the control group. These findings might be attributed to changes in seminiferous tubules, interstitial cells, and the spermatogenesis process as previously reported (Raeeszadeh *et al*., 2021; Rauf *et al*., 2021). Furthermore, the changes might be attributed to an alteration in the TBF as previously reported (El-Shalofy *et al*., 2021). From the foregoing, we might be provided with an explanation for the changes in echogenicity.

Testosterone hormone is the primary hormone responsible for fetal male sexual differentiation, pubertal development, spermatogenesis maintenance, and prevention of germ cell apoptosis (Chandra *et al*., 2007, 2010). Vitamin C is involved in the synthesis of sex steroids such as testosterone because the hydroxylation of steroids is vitamin C-dependent (Luck *et al*., 1995; Weber *et al*., 1996). Although increased testicular blood flow corresponds to an increase in testosterone levels as previously reported in goats (Strina *et al*., 2016), there was no change in T concentrations in our study. Our findings were contrary to the previously reported in adult male rats (Rauf *et al*., 2021), and this variation in response could be attributed to differences in animal species, animal age, and method of nanoparticle preparation (Saki *et al*., 2013).

The increased free radical activity is associated with several conditions, including HS, and consumes radical scavenging antioxidants. Total antioxidant capacity (TAC) assay provides a method for measuring the combined antioxidant activities exerted by both enzymatic and non-enzymatic seminal plasma antioxidants (Barranco *et al*., 2015). Vitamin C is one of the non-enzymatic antioxidants that need to be assessed. In the current work, there was an increase in TAC values after vitamin C and its NPs administration. Our results were consistent with a previous study (Moradi *et al*., 2024) in mice. To definitely explain the TAC increases, further detailed analysis for different antioxidants is required.

Conclusion

Collectively, vitamin C NPs improve testicular vascularization and TV than traditional vitamin C. Pubsecnt bucks administered the vitamin C in its nano-formulations showed higher concentrations of NO compared to traditional vitamin C and the control group. So, the administration of nanoparticles of vitamin C could be recommended for improving the reproductive performance of pubescent bucks under HS conditions and treating more animals with a low dose compared to the conventional dose.

Acknowledgments

The authors extend their appreciation to the staff members of the Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Egypt for allowing using animals.

Conflict of interest

The authors have no conflict of interest to declare.

References

Abbas, M., Khan, M.I.U.R., Hameed, N., Rehman, A., Mohsin, I., Bilal, M., Shahzad, M., 2021. Melatonin along with eCG improves fresh semen quality and plasma concentrations of melatonin and testosterone during non-breeding season in Beetal bucks. Small Rumin Res. 205, 106-569. https://doi.org/10.1016/j.smallrumres.2021.106569

Abdelkhalek, K.G., Badawy, A.B.A., Fathi, M., Abdelnaby, E.A., 2022. Reproductive hormonal levels and nitric oxide levels as guides of pubertal reproductive development in relation to testicular width and hemodynamics in Baladi bucks. Adv Anim Vet Sci. 10, 236-243. http://doi.org/10.17582/journal.aavs/2022/10.2.236.243

Abdelnaby, E.A., Emam, I.A., Fadl, A.M., 2021. Assessment of the accuracy of testicular dysfunction detection in male donkey (*Equus asinus*) with the aid of colour-spectral Doppler in relation to plasma testosterone and serum nitric oxide levels. Reprod Domest Anim. 56, 764-774. https://doi.org/10.1111/rda.13916

AHmadi, B., Lau, C.P.S., Giffin, J., Santos, N., Hahnel, A., Raeside, J., Bartlewski, P., 2012. Suitability of epididymal and testicular ultrasonography and computerized image analysis for assessment of current and future semen quality in the ram. Experimental Biology and Medicine 237, 186-193. https://doi.org/10.1258/ebm.2011.011050

Al-Dawood, A., 2017. Effect of heat stress on adipokines and some blood metabolites in goats from Jordan. Anim. Sci. J. 88, 356–363.

Alishahi, A., Mirvaghefi, A., Tehrani, M.R., Farahmand, H., Shojaosadati, S.A., Dorkoosh, F.A., Elsabee, M.Z., 2011. Shelf life and delivery enhancement of vitamin C using chitosan nanoparticles. Food Chem. 126, 935–940.

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., Chang, C. M., 2022. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules 27, 1326. doi: 10.3390/molecules27041326.

Bansal, A.K., Bilaspuri, G.S., 2011. Impacts of oxidative stress and antioxidants on semen functions. Vet Med Int, 2011, 686137. https://doi.org/10.4061/2011/686137

Barranco, I., Tvarijonaviciute, A., Perez-patiño, C., Parrilla, I., Ceron, J.J., Martinez, E.A., Roca, J., 2015. High total antioxidant capacity of the porcine seminal plasma (SP-TAC) relates to sperm survival and fertility. Scientific Reports. 5, 18538. https://doi.org/10.1038/srep18538

Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A., 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J Dairy Sci. 85, 2173-2179. https://doi.org/10.3168/jds.S0022-0302(02)74296-3

Biagiotti, G., Cavallini, G., Modenini, F., Vitali, G., Gianaroli, L., 2002. Spermatogenesis and spectral echo-colour Doppler traces from the main testicular artery. BJU Int. 90, 903-908. https://doi.org/10.1046/j.1464-410X.2002.03033

Brito, L.F.C., Barth, A.D., Wilde, R.E., Kastelic, J.P., 2012. Testicular ultrasonogram pixel intensity during sexual development and its relationship with semen quality, sperm production, and quantitative testicular histology in beef bulls. Theriogenology 78, 69-76. https:// doi. org/10. 1016/j. theriogenology. 2012. 01.022

Bumin, A., Kaya, M.A.H.İ.R., Kaya, Ü., Kibar, M., Alkan, Z., 2007. Gray-scale, colour and power Doppler sonography of scrotal disorders in dogs. Rev. Med. Vet. 158, 128-133.

Camela, E.S., Nociti, R.P., Santos, V.J., Macente, B.I., Murawski, M., Vicente, W.R., Oliveira, M.E.F., 2019. Changes in testicular size, echotexture, and arterial blood flow associated with the attainment of puberty in Dorper rams raised in a subtropical climate. Reprod. Domest. Anim. 54, 131–137. https://doi.org/10.1111/rda.13213

Chae, C.H., Park, J.W., 2007. The study on the effect of nanoemulsion for the prevention and treatment of gingival inflammation. J. Korean Assoc. Oral Maxillofac. Surg. 33, 419–425.

Chandra, A.K., Chatterjee, A., Ghosh, R., Sarkar, M., 2010. Vitamin E-supplementation protect chromium (VI)-induced spermatogenic and steroidogenic disorders in testicular tissues of rats. Food Chem. Toxicol. 48, 972-979] https://doi.org/10.1016/j.fct.2010.01.008

Chandra, A.K., Chatterjee, A., Ghosh, R., Sarkar, M., Chaube, S.K., 2007. Chromium induced testicular impairment in relation to adrenocortical activities in adult albino rats. Reprod. Toxicol. 24, 388-396] https://doi.org/10.1016/j.reprotox.2007.07.009

Comunian, T., Babazadeh, A., Rehman, A., Shaddel, R., Akbari-Alavijeh, S., Boostani, S., Jafari, S.M., 2022. Protection and controlled release of vitamin C by different micro/nanocarriers. Crit. Rev. Food Sci. Nutr. 62, 3301-3322.

Daykin, P.W., 1960. Veterinary applied pharmacology and therapeutics. London: Bailliere Tindall and Cassell. 619–621.

Dickey, R.P., 1997. Doppler ultrasound investigation of uterine and ovarian blood flow in infertility and early pregnancy. Hum Reprod. Update 3, 467–503.

Dudea, S.M., Ciurea, A., Chiorean, A., Botar-Jid, C., 2010. Doppler applications in testicular and scrotal disease. Med. Ultrason. 12, 43-51.

Ebesunun, M.O., Solademi, B.A., Shittu, O.B., Anetor, J.I., Onuegbu, J.A., Olisekodiaka, J.M., Onyeaghala, A.A., 2004. Plasma and semen ascorbic levels in spermatogenesis. West Afr. J. Med. 23, 290–293.

El Tohamy, M.M., Kotp, M.S., El Nattat, W.S., Mohamed, A.H., 2012. Semen characteristics and oxidative/antioxidative status in semen and serum of male rabbits supplemented with antioxidants during heat stress. Iran. J. Appl. Anim. Sci. 2, 175–183.

El-Shalofy, A., Hedia, M., Kastelic, J., 2021. Melatonin improves testicular haemo-

dynamics, echotexture and testosterone production in Ossimi rams during the breeding season. Reprod. Domest. Anim. 56, 1456-1463. https://doi.org/10.1111/rda.14010

El-Sherbiny, H.R., Abdelnaby, E.A., El-Shahat, K.H., Salem, N.Y., Ramadan, E.S., Yehia, S.G., Fathi, M., 2022a. Coenzyme Q10 Supplementation enhances testicular volume and hemodynamics, reproductive hormones, sperm quality, and seminal antioxidant capacity in goat bucks under summer hot humid conditions. Vet. Res. Com¬mun. 46, 1245-1257. https://doi.org/10.1007/s11259-022-09991-8

EL-Sherbiny, H.R., EL-Shalofy, A.S., Samir, H., 2022b. Exogenous L-carnitine administration ameliorates the adverse effects of heat stress on testicular hemodynamics, echotexture, and total antioxidant capacity in rams. Front. Vet. Sci. 9:860771. https://doi.org/10.3389/fvets.2022.860771

EL-Tarabany, M.S., EL-Tarabany, A.A., Atta, M.A., 2017. Physiological and lactation responses of Egyptian dairy Baladi goats to natural thermal stress under subtropical environmental conditions. Int. J. Biometeorol. 61, 61–68. https:// doi.org/ 10. 1007/ s00484- 016- 1191-2

Englard, S., Seifter, S., 1986. The biochemical functions of ascorbic acid. Annu. Rev. Nutr. 6, 365–406.

Fazeli, P., Zamiri, M.J., Farshad, A., Khalili, B., 2010. Effects of vitamin C on testicular and seminal characteristics of Markhoz goats. Iranian Journal of Veterinary Research, Shiraz University 11, 267-297

Fraga, C.G., Motchnik, P.A., Shigenaga, M.K., Helbock, H.J., Jacob, R.A., AMES, B.N., 1991. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Proc. Natl. Acad. Sci. U.S.A. 88, 11003-11006.

Galal, S., Abdel-Rasoul, F., Shaat, I., Anous, M., 2005. On-station characterization of small ruminant breeds in Egypt. Characterization of Small Ruminant Breeds in West Asia and North Africa. Luis Inigez, editor. Aleppo; 2005. https://scholar.google.com/citations?view_op=view_citation&hl=en&user=FMLU8PMAAAAJ&citation_for_view=FMLU8PMAAAAJ:2osOgNQ5qMEC.

Ginther, O.J., 2007. Ultrasonic imaging and animal reproduction: Color-Doppler ultrasonography—Book 4. Cross plains: Equiservices Publishing 2007, p.7-81.

Gloria, A., Di Francesco, L., Marruchella, G., Robbe, D., Contri, A., 2020. Pulse-wave Doppler pulsatility and resistive indexes of the testicular artery increase in canine testis with abnormal spermatogenesis. Theriogenology 158, 454-460. https://doi.org/10.1016/j.theriogenology.2020. 10.015

Gopi, S., Balakrishnan, P., 2021. Evaluation and clinical comparison studies on liposomal and non-liposomal ascorbic acid (vitamin C) and their enhanced bioavailability. J. liposome Res. 31, 356-364.

Gumbsch, P., Holzmann, A., Gabler, C., 2002. Colour-coded duplex sonography of the testes of dogs. Veterinary Record 151, 140-144. https://doi.org/10.1136/vr.151.5.140

Günzel-Apel, A.R., Möhrke, C., Nautrup, C.P., 2001. Colour-coded and pulsed Doppler sonography of the canine testis, epididymis and prostate gland: Physiological and pathological findings. Reprod Domest Anim. 36, 236-240.

Hansen, P.J., 2009. Effects of heat stress on mammalian reproduction. Philos Trans R Soc Lond B Biol Sci. 364, 3341–33450. https://doi.org/10.1098/rstb.2009.0131

Hedia, M.G., EL-Belely, M.S., Ismail, S.T., Abo El-Maaty, A.M., 2020. Seasonal variation in testicular blood flow dynamics and their relation to systemic and testicular oxidant/antioxidant biomarkers and androgens in rams. Reprod. Domest. Anim. 55, 861-869. https://doi.org/10.1111/rda.13696

Herbig, A.L., Renard, C.M., 2017. Factors that impact the stability of vitamin C at intermediate temperatures in a food matrix. Food Chem. 220, 444-451.

Kay, G.W., Grobbelaar, J.A.N., Hattingh, J., 1992. Effect of surgical restriction of growth of the testicular artery on testis size and histology in bulls. Reproduction 96, 549-553.

Kendall, P., Webster, J., 2009. Season and physiological status affects the circadian body temperature rhythm of dairy cows. Livest. Sci. 125,155–160.

Khalili, A., Alipour, S., Fathalipour, M., Purkhosrow, A., Bayat, G., Nekooeian, A.A., 2020. Liposomal and non-liposomal formulations of vitamin C: Comparison of the antihypertensive and vascular modifying activity in renovascular hypertensive rats. Iran. J. Med. Sci. 45, 41-49.

Lissbrant, E., Löfmark, U., Collin, O., Bergh, A., 1997. Is nitric oxide involved in the regulation of the rat testicular vasculature? Biol Reprod. 56, 1221-1227. https://doi.org/10.1095/biolreprod56.5.1221

Lu, C.D., 1989. Effects of heat stress on goat production. Small Rumin Res. 2, 151-162. https://doi.org/10.1016/0921-4488(89)90040-0

Luck, M.R., Jeyaseelan, I., Scholes, R.A., 1995. Ascorbic acid and fertility. Biol. Reprod. 52, 262-266.

Marai, I.F.M., Abou-Fandoud, E.I., Daader, A.H., Abu-Ella, A.A., 2002. Reproductive doe traits of the Nubian (Zaraibi) goats in Egypt. Small Rumin Res. 46, 201-205. https://doi.org/10.1016/S0921-4488(02)00195-5

Mikirova, N., Levy, T., Hunninghake, R., 2019. The levels of ascorbic acid in blood and mononuclear blood cells after Oral liposome-encapsulated and Oral non-encapsulated vitamin C supplementation, taken without and with IV hydrocortisone. J Orthomol Med. 34, 2019

Minka, N.S., Ayo, J.O., 2010. Physiological responses of erythrocytes of goats to transportation and the mondulatory role of ascorbic acid. J Vet Med Sci. 72, 875-881.

Minka, N.S., Ayo, J.O., 2012. Assessment of thermal load on transported goats administered with ascorbic acid during the hot-dry conditions. Int J Biometerol. 56, 333-341.

Montes-Garrido, R., Anel-Lopez, L., Riesco, M.F., Neila-Montero, M., Palacin-Martinez, C., Soriano-Úbeda, C., Alvarez, M., 2023. Does Size Matter? Testicular Volume and Its Predictive Ability of Sperm Production in Rams. Animals 13, 3204.

Moradi, M., Hashemian, M.A., Fathi, M., Peysokhan, M., Hashemian, A.H., Moradi, B., Faramarzi, A., 2024. Utility of vitamin C in ameliorating citalopram-induced testicular toxicity via modulating nitro-oxidative stress and apoptosis in mice. J Biochem. Mol. Toxicol. 38, 23543.

Parhizkar, E., Rashedinia, M., Karimi, M., Alipour, S., 2018. Design and development of vitamin C-encapsulated proliposome with improved in-vitro and ex-vivo antioxidant efficacy. J. Microencapsul. 35, 301-311.

Paul, C., Murray, A. A., Spears, N., Saunders, P.T., 2008. A single, mild, transient scrotal heat stress causes DNA damage, subfertility and impairs formation of blastocysts in mice. Reproduction 136, 73–84. https://doi.org/10.1530/ REP-08-0036

Paulis, L., Šimko, F., 2007. Blood pressure modulation and cardiovascular protection by melatonin: potential mechanisms behind. Physiol. Res. 56, 671-684. https://doi.org/10.33549/physiolres.931236

Phillips, P.H., Lardy, H.A., Boyer, P.D., Werner, G.M., 1941. The relationship of ascorbic acid to reproduction in the cow. J. Dairy Sci. 24, 153-158.

Phillips, P.H., Lardy, H.A., Heizer, E.E., Rüpel, I.W., 1940. Sperm stimulation in the bull through the subcutaneous administration of ascorbic acid. J. Dairy Sci. 23, 873-878.

Pozor, M., Morrissey, H., Albanese, V., Khouzam, N., Deriberprey, A., Macpherson, M. L., Kelleman, A.A., 2017. Relationship between echotextural and histomorphometric characteristics of stallion testes. Theriogenology 99, 134-145. https://doi.org/10.1016/j.theriogenology.2017.05.031

Pozor, M.A., Mcdonnell, S.M., 2004. Color Doppler ultrasound evaluation of testicular blood flow in stallions. Theriogenology 61, 799-810. https://doi.org/10.1016/S0093-691X(03)00227-9

Raeeszadeh, M., Karimfar, B., Amiri, A.A., Akbari, A., 2021. Protective Effect of Nano-Vitamin C on Infertility due to Oxidative Stress Induced by Lead and Arsenic in Male Rats. J. Chem. 2021, 9589345.

Ralston, S.L., Barbacini, S., Squires, E.L., Nockels, C.F., 1988. Ascorbic acid supplementation in stallions. J. Equine Vet. Sci. 8, 290-293.

Ranjan, R., Ranjan, A., Dhaliwal, G.S., Patra, R.C., 2012. L-Ascorbic acid (vitamin C) supplementation to optimize health and reproduction in cattle. Veterinary Quarterly 32, 145-150.

Rauf, N., Nawaz, A., Ullah, H., Ullah, R., Nabi, G., Ullah, A., FU, J., 2021. Therapeutic effects of chitosan-embedded vitamin C, E nanoparticles against cisplatin-induced gametogenic and androgenic toxicity in adult male rats. Environ. Sci. Pollut. Res. 28, 56319-56332.

Ribeiro, D.L.D.S., Santos, L.S., França, I.G., Pereira, H.G., Lima, J.D.S., Miranda, B.D.B., Junior, J.R.S.T., 2020. The use of Doppler ultrasound as a potential fertility predictor in male goats. Turk. J. Vet. Anim. Sci. 44,1115-1124. https://doi.org/10.3906/vet-2003-135

Saaed, F.F., Zaid, N.W., 2018. Predictor ultrasonographic evaluation of the testis during pubertal age in ram lambs. Adv. Anim. Vet. Sci. 6, 521-525.

Sakamoto, H., Ogawa, Y., Yoshida, H., 2008. Relationship between testicular volume and testicular function: comparison of the Prader orchidometric and ultrasonographic measurements in patients with infertility. Asian J. Androl. 10, 319–324. https://doi.org/10.1111/j.1745-7262.2008.00340.

Saki, G., Jasemi, M., Sarkaki, A.R., Fathollahi, A., 2013. Effect of administration of vitamins C and E on fertilization capacity of rats exposed to noise stress. Noise and Health 15, 194-198.

Samir, H., Nyametease, P., Nagaoka, K., Watanabe, G., 2018. Effect of seasonality on testicular blood flow as determined by color Doppler ultrasonography and hormonal profiles in Shiba goats. Anim. Reprod. Sci. 197, 185-192. https://doi.org/10.1016/j.anireprosci.2018.08.027

Samir, H., Sasaki, K., Ahmed, E., Karen, A., Nagaoka, K., El Sayed, M., Taya, K., Watanabe, G., 2015. Effect of a single injection of gonadotropin-releasing hormone (GnRH) and human chorionic gonadotropin (hCG) on testicular blood flow measured by color doppler ultrasonography in male Shiba goats. J. Vet. Med. Sci. 77, 549–556. https://doi.org/10.1292/jvms.14-0633

Sarlós, P., Egerszegi, I., BalOGH, O., Molnár, A., Cseh, S., Rátky, J., 2013. Seasonal changes of scrotal circumference, blood plasma testosterone concentration and semen characteristics in Racka rams. Small Rumin. Res. 111, 90-95. https://doi.org/10.1016/j.smallrumres.2012.11.036

Schwartz-duval, A.S., Konopka, C.J., Moitra, P., Daza, E.A., Srivastava, I., Johnson, E.V., Pan, D., 2020. Intratumoral generation of photothermal gold nanoparticles through a vectorized biomineralization of ionic gold. Nat. Commun. 11, 4530.

Shadman, R., Ziaei, N., Esmaeilipour, O., Ranjbar, M., 2022. Comparison the effects of vitamin C with nano vitamin C supplement on growth performance, immune responses, intestine morphology and blood parameters of heat stressed broiler chickens. Anim Sci Res. 32, 71-83.

Shahat, A.M., Rizzoto, G., Kastelic, J.P., 2020. Amelioration of heat stress-induced damage to testes and sperm quality. Theriogenology 158, 84-96. https://doi.org/10.1016/j.theriogenology.2020.08.034

Sikka, S.C., 1996. Oxidative stress and role of antioxidants in normal and abnormal sperm function. Frontiers BioSci. 1, 78-86.

Silva, H. D., Cerqueira, M. A., Souza, B. W. S., Ribeiro, C., Avides, M. C., Quintas, M. A, Vicente A.A., 2011. Nanoemulsions of β-carotene using a high-energy emulsification–evaporation technique. J. Food Eng. 102, 130-135. https://doi.org/10.1016/j.jfoodeng.2010.08.005

Strina, A., Corda, A., Nieddu, S., Solinas, G., Lilliu, M., Zedda, M. T., Ledda, S., 2016. Annual variations in resistive index (RI) of testicular artery, volume measurements and testosterone levels in bucks. Comp. Clin. Path. 25, 409-413.

Subroto, E., Putri, N.A.G., Rahmani, F.R., Nuramalia, A.F., Musthafa, D.A., 2021. Bioavailability and Bioactivity of Vitamin C-A Review. Int. J. Pharm. Res. 13, 1-16

Takihara, H., Sakatoku, J., Fujii, M., Nasu, T., Cosentino, M. J., Cockett, A.T., 1983. Significance of testicular size measurement in andrology. I. A new orchiometer and its clinical application. Fertil. Steril. 39, 836–840. https://doi.org/10.1016/S0015-0282(16)4712-8

Teama, F.E.I., EL-Tarabany, A.A., 2016. Physiological and biochemical response to Omega-3 plus as a dietary supplement to growing goats under hot summer conditions. Rev. Bras de Zootec. 45,174–180. https:// doi. org/ 10. 1590/ S1806-92902 01600 04000 05

Weber, P., Bendich, A., Schalch, W., 1996. Vitamin C and human health--a review of recent data relevant to human requirements. International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin-und Ernahrungsforschung. Int. J. Nutr. Res. 66, 19-30.

Zhao, P., Ruiz, J., Salmon, L., Astruc, D., 2012. Encapsulation of Water-Soluble Vitamins by Gold Nanoparticles in Hydrophobic Media. Chemistry Letters. 41, 1107-1109. https://doi.org/10.1246/cl.2012.1107