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(Original Article)

Effect of Pomegranate Juice Supplementation on Reproductive Performance, Oxidative Status, and Testosterone Level in New Zealand Male Rabbits

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Abstract

This study evaluated the effects of pomegranate juice (PJ) supplementation on reproductive performance, oxidative stress markers, and testosterone levels in male New Zealand rabbits. Twelve rabbits were randomly divided into two groups: A control group receiving plain tap water and a treatment group supplemented with 20 mL of PJ daily for a period of 2 months (3rd of January to 28th of February 2021). Key reproductive parameters, including mount latency, semen quality, sperm motility and viability, and circulating testosterone levels were assessed. Additionally, serum anti-oxidative markers such as total antioxidant capacity (TAC), malondialdehyde (MDA), and catalase (CAT) activity were measured.

The results demonstrated that PJ supplementation significantly ($P<0.05$) reduced mount latency and increased testosterone levels, ejaculate volume and total sperm motility compared to the control group. While sperm concentration per milliliter decreased ($P<0.05$) and the total sperm count remained unaffected. Serum analysis revealed that rabbits receiving PJ exhibited higher TAC and reduced MDA concentrations ($P<0.05$), indicating improved oxidative status. No significant changes were observed in catalase (CAT) activity.

These findings suggest that PJ can enhance reproductive performance and mitigate oxidative stress, potentially by supporting antioxidant defenses and testosterone production. PJ's antioxidant-rich profile, including polyphenols and punicalagins, may contribute to its beneficial effects on male fertility, supporting its use in breeding programs.

Keywords: *Polyphenols, Redox status, Sexual performance, Sperm kinematics, Sperm quality.*

Introduction

Rabbits (*Oryctolagus cuniculus*) play an essential role across various sectors, including meat production and biomedical research. Their rapid maturity, high reproductive capacity, and brief gestation make them favorable for small-scale farming and commercial breeding (Galal and Khalil, 1994; Ajayi *et al.,* 2005). These qualities allow for efficient production cycles. However, male fertility in rabbits remains vulnerable to environmental challenges, particularly oxidative stress, which has become

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a major concern due to its detrimental effects on sperm function and reproductive outcomes (Attia *et al.,* 2017).

Oxidative stress results from an imbalance between reactive oxygen species (ROS) and the body's ability to counteract or detoxify them through antioxidants. Although ROS are normal byproducts of metabolism, their excessive accumulation can damage lipids, proteins, and DNA (Donnelly *et al.,* 2001). Rabbit sperm cells are especially prone to oxidative injury because their membranes are rich in polyunsaturated fatty acids (PUFAs), which are highly susceptible to peroxidation (Am-in *et al.,* 2010). Left unchecked, oxidative stress impairs motility, concentration, and membrane integrity of sperm, reducing fertility (Lombardo *et al.,* 2011).

To counteract oxidative damage, interest has grown in using natural antioxidants, which help maintain sperm function by scavenging free radicals. Pomegranate (*Punica granatum*), known for its robust antioxidant profile, has emerged as a promising dietary supplement in this context. Rich in polyphenols, tannins, flavonoids, and punicalagin, pomegranate exhibits remarkable capacity to neutralize ROS (Fischer *et al.,* 2011). These bioactive compounds, particularly concentrated in the peel, offer potent protective effects against oxidative stress, safeguarding cellular components from lipid and protein damage (Elfalleh *et al.,* 2012).

Experimental studies have highlighted pomegranate's potential to enhance male fertility by improving key semen parameters such as sperm motility, viability, and concentration. The antioxidants found in pomegranate, including punicalagin and ellagic acid, boost the activity of enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Sun *et al.,* 2017). This enzymatic defense helps reduce lipid peroxidation and preserves the functional integrity of sperm cells (Bakeer *et al.,* 2021).

Research involving rabbits has shown significant improvements in semen quality following dietary supplementation with pomegranate extracts. Zeweil *et al.* (2013) reported that incorporating pomegranate peel powder into rabbit feed led to higher sperm concentration, better motility, and fewer abnormal sperm. Similarly, Fayed *et al.* (2012) found that feeding pomegranate extracts increased antioxidant enzyme levels in seminal plasma while reducing oxidative stress markers such as malondialdehyde (MDA). These findings suggest that pomegranate peel may serve as an effective natural supplement for enhancing reproductive performance in rabbits.

Pomegranate's effects are not limited to semen quality; it also plays a role in hormone regulation, particularly testosterone. Elevated testosterone levels are essential for maintaining spermatogenesis, sexual behavior, and reproductive performance (Qasim and Ali ,2020). The antioxidant properties of pomegranate support testosterone production by protecting Leydig cells, key players in androgen synthesis, from oxidative damage. Furthermore, pomegranate has shown potential to enhance libido and sexual behavior by improving blood flow and supporting erectile function (Al-Dujaili and Smail, 2012).

This study seeks to examine the impact of pomegranate juice supplementation on the reproductive health of male rabbits. It investigates whether pomegranate juice

influences redox status, circulating testosterone, and key sperm parameters, including motility and viability, as well as its effects on testicular tissues. The findings aim to provide insights into pomegranate's potential as a natural antioxidant for improving reproductive outcomes and its application in rabbit breeding programs.

Materials and Methods

Study Location

The experiment was conducted during the period from the 3rd of January to 28th of February 2021. This experiment was conducted at the Poultry Research Farm, Faculty of Agriculture, Assiut University, Upper Egypt. The study aimed to evaluate the impact of pomegranate juice supplementation in drinking water on the reproductive performance of male New Zealand rabbits.

Animals

A total of 12 male New Zealand rabbits were used in the study. All rabbits were 8 months old, with an average body weight of 3.24 kg.

Feeding Regimen

All rabbits were fed a basal diet provided 2100 kcal/kg and contained 12 % crude protein, 14% crude fiber and 2 % fat, meeting the nutritional standards outlined by the Nutrient Requirements of rabbits (NRR, 1977). Throughout the experiment, animals had free access to both feed and water.

Water was supplied through a nipple drinker system installed in the housing units, and experimental treatments were administered through water tanks according to the designated groups.

Housing

All rabbits were individually housed in galvanized wire cages, measuring 60 × 50 × 35 cm. Each animal was provided with consistent managerial, nutritional, and hygienic care to ensure uniform conditions throughout the study.

Environmental parameters were maintained in accordance with animal welfare guidelines. The facility was kept at a controlled temperature of 23°C, with relative humidity (RH) ranging between 62-68%. A 12-hour light/12-hour dark cycle was implemented to simulate natural photoperiods.

Pomegranate Juice Preparation

Pomegranate fruits (*Punica granatum* var. Manfaloty) were sourced from a commercial market in Manfalut, Assiut, Egypt, and transported directly to the laboratory. Upon arrival, fruits were inspected, and any with defects—such as sunburn, cracks, bruises, or cuts—were discarded.

The selected fruits were washed thoroughly with tap water and drained. Each fruit was quartered, and the peel and seeds were manually separated to prepare the juice. All processing was carried out immediately to preserve the fruits' bioactive compounds for the experiment.

The seeds were rinsed under running water to remove residues from the peels. They were then blended in an electric mixer (Fresh Blender, 360 Watt) for 30 seconds. The mixture was passed through a cheesecloth to separate rind particles, yielding fruit juice. The discarded seeds were not used further.

The extracted juice was centrifuged at 3000 rpm for 5 minutes, and the resulting supernatant was collected for analysis of total phenolic content. The juice was stored at -20°C until further use.

Determination of Total Polyphenol Content in Pomegranate Juice (PJ)

The total phenolic content (TPC) of pomegranate juice (PJ) was determined using the Folin–Ciocalteu method (Singleton and Rossi, 1965). To prepare stock solutions, 100 µL of juice was mixed with 1 mL of deionized water. From each stock solution and a blank, 300 µL was pipetted into separate test tubes, followed by the addition of 300 µL of Folin–Ciocalteu reagent. The mixtures were thoroughly mixed and allowed to equilibrate for 2 minutes.

After equilibration, 2.4 mL of a 5% (w/v) sodium carbonate solution was added to each tube, and the contents were gently swirled. The tubes were then incubated in a water bath at 40°C for 20 minutes. Once the incubation was complete, the tubes were quickly cooled to room temperature, and the absorbance was measured at 740 nm using a spectrophotometer (UNICAM BS DISC PD 2000-1).

The TPC values were expressed as gallic acid equivalents (GAE) based on a gallic acid calibration curve (Ferrara *et al.,* 2011).

Experimental Design

The rabbits were randomly assigned to two groups:

Group 1 (Control): Rabbits were given plain tap water without any additives.

Group 2 (Treatment): Each rabbit received 20 mL of pomegranate juice (PJ) daily, containing total phenols equivalent to 0.66 mg of ellagic acid per mL.

Evaluation of Bucks' Reproductive Performance

Mount latency and semen collection

Experimental observations were conducted over a period of 8 weeks, during which female rabbits were introduced to the males once a week, and the reaction time, defined as the interval from the introduction of a "teaser" doe into the male's cage to the first mounting with vigorous thrust, served as an indicator of libido. Semen was collected once a week (8 times in total) using an artificial vagina (IMV Technologies, France), and gel plugs from ejaculates were removed. Each ejaculate was stored in a water bath maintained at 37°C for up to 15 minutes before evaluation. The volume of each ejaculate was recorded using a graduated collecting tube.

Assessment of Sperm Viability

The viability of spermatozoa was assessed using eosin-nigrosin staining, following the protocol described by Björndahl *et al.* (2003). An aliquot of semen was mixed with an equal volume of eosin-nigrosin suspension, and a smear was prepared on a glass slide.

Once the smear dried, 100 spermatozoa in each replicate were examined at 400X magnification using a microscope and counted with a laboratory counter. Live spermatozoa exhibited white heads, while dead spermatozoa displayed red or dark pink heads.

Sperm Kinematics

Sperm motility and velocity parameters were analyzed using a computer-assisted sperm analysis (CASA) system (Mira-9000, Sperm Analyzer CASA software, Mira Lab, Egypt). Before analysis, the system was calibrated to match the morphometric characteristics of rabbit sperm. The software settings were as follows: frames per second (fps) = 30, minimum contrast = 50, minimum data points = 10, static sperm threshold (μm/sec) < 10, minimum cell size = 5 pixels, and thresholds set at 20 for average path velocity (VAP) and 70% for straightness (STR).

For motility assessment, a semen sample diluted to 25 × 10^6 sperm/mL with a Tris-citrate-glucose extender was used. A 10 μL drop was placed on a pre-warmed CASA spermolyzer metal slide chamber (10 μm depth), covered with a CASA coverslip, and analyzed on a heated microscope stage maintained at 37°C. Each sample was examined under a phase-contrast microscope (Leica, Germany) at 20× magnification. A minimum of ten fields and 500 sperm tracks per sample were evaluated using the CASA software (Spermolyzer, Mira Lab, Egypt).

The motility parameters assessed included total motility (TMOT, %), progressive motility (PR, %), non-progressive motility (NP, %; indicating motility without progression), and immotility (IM, %; the proportion of non-motile sperm). Additionally, kinematic parameters measured included curvilinear velocity (VCL, μm/s), straight-line velocity (VSL, μm/s), average path velocity (VAP, μm/s), linearity (LIN, %; calculated as the ratio of VSL to VCL), straightness (STR, %; the ratio of VSL to VAP), amplitude of lateral head displacement (ALH, μm; the extent of head oscillation during movement), mean angular displacement (MAD; the average turning angle of the sperm head), and beat cross-frequency (BCF, Hz; the frequency at which the sperm head crosses its average path in either direction).

Evaluation of Testosterone Levels

At the conclusion of the experiment (60 days post-treatment), blood samples (2 mL from each rabbit) were withdrawn from marginal ear vein and collected in tubes without anticoagulant. The blood was allowed to coagulate in an incubator at 37°C and subsequently centrifuged at 3000 rpm for 5 minutes. Serum was harvested into sterile, dry Eppendorf tubes and stored at -20°C until analysis.

Serum levels of testosterone (T) were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Biosource Europe S.A, Belgium). This competitive ELISA utilized an antibody and testosterone-streptavidin–horseradish peroxidase (HRP) conjugate. Samples and standards were incubated with the testosterone-HRP conjugate in pre-coated plates with anti-testosterone antibody for 1 hour at 37°C. After incubation, the mixtures were decanted, and the wells were washed five times with wash buffer. The plate was then incubated with HRP substrate for 15 minutes at 37°C, resulting in the formation of a blue-colored complex. A stop solution was added to halt

the reaction, converting the blue complex to yellow. The intensity of this color was measured calorimetrically at 450 nm using a microplate reader. The intensity of the color was inversely proportional to testosterone concentration, as testosterone in the samples competed with the testosterone-HRP conjugate for binding to the anti-testosterone antibody (Hasan *et al*., 2020).

Determination of Redox status

All blood plasma biochemical variables and redox status indicators were analyzed using spectrophotometric procedures with a Hitachi spectrophotometer (Japan) and commercial kits sourced from Biodiagnostic (Giza, Egypt).

Malondialdehyde (MDA)

The concentration of malondialdehyde (nmol/mL) in serum samples was assessed according to the method described by Ohkawa (1978). A kit from Bio-Diagnostic Company was used, where MDA reacts directly with thiobarbituric acid at an optimal pH of 3.5 to produce a red-colored complex, which is measured spectrophotometrically.

Catalase (CAT)

Serum catalase activity (nmol/dL) was determined following the procedure outlined by Aebi (1984), using a kit from Bio-Diagnostic Company. In this assay, the remaining hydrogen peroxide (H_2O_2) reacts with 3,5-Dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) in the presence of horseradish peroxidase (HRP) to form a chromophore. The intensity of the color produced is inversely proportional to the amount of catalase present in the original serum sample.

Total Antioxidant Capacity (TAC)

Total antioxidant capacity (mmol/L) in serum was determined using a colorimetric method. This involved measuring the reaction of oxidants in the serum with a defined amount of oxygen, which typically produces hydrogen peroxide (H_2O_2). The residual H_2O_2 was quantified colorimetrically by analyzing the enzymatic colored product. TAC was calculated by subtracting the absorbance of the blank from that of the sample and multiplying by 3.33. The absorbance readings for both the blank and the samples were taken against deionized water at wavelengths of 500-510 nm.

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) by applying the general linear model procedure of SAS, 2009 software statistical programs. All means were tested for significant differences using Duncan‘s multiple range procedure (Duncan, 1955).

The following statistical model was used for ANOVA: $Yij = \mu + Ti + eij$,

Yij = an observation, μ = the overall mean, Ti = treatment effect and eij = experimental random error. The replicate was the experimental unit in the present work.

Results

The effects of pomegranate juice supplementation on mount latency and semen characteristics are summarized in Table 1.

Supplementation via drinking water significantly reduced mount latency and increased ejaculate volume ($P < 0.001$) compared to the control group. While sperm concentration per milliliter decreased in the pomegranate juice-treated group, no significant differences were observed in the total ejaculate sperm count. Additionally, live and dead sperm counts remained comparable across all groups.

Table 1. Effect of oral administration of juice on the reaction time, semen volume, sperm concentration, total sperm concentration, viable sperms, and Dead sperms of New Zealand Rabbit bucks (mean)

Traits	Control	Juice	SEM	P-value
Mount latency (Sc)	11.21^{a}±0.70	4.25^{b}±0.67	0.53	<0.0001
Semen volume (ml/ej)	0.74^{b}±0.01	0.82^{a}±0.01	0.009	0.0029
Sperm concentration ($\times10^{6}$/mm3)	180.50^{a}±2.11	158.00^{b}±0.63	1.525	0.0005
Total sperm concentration ($\times10^{6}$/ejaculate)	132.65±1.73	129.45±1.29	1.899	0.2987
Viable sperms (%)	78.92±1.88	83.15±0.95	1.934	0.1973
Dead sperms (%)	21.10±1.86	16.85±0.95	1.919	0.1932

$^{a-b}$ means within a row with different superscripts differ significantly ($P<0.05$). Control: Plan water, Juice: 13.2 mg total phenols from the pomegranate Juice/rabbits. Number of samples =12

The effects of pomegranate juice administration via drinking water on sperm motility and kinematics are summarized in Table 2.

Pomegranate juice consumption significantly improved total fresh sperm motility compared to the control group, but no significant differences were detected in any sperm kinematic parameters.

Table 2. Effect of oral administration of aqueous pomegranate Juice on the sperm kinematics (motility and velocity) of New Zealand Rabbit bucks (mean)

Traits		Control	Juice	SEM	P-value
Motility	**TMOT %**	92.7^{b}±0.55	94.3^{a}±0.46	0.398	0.0040
	PR %	27.9^{b}±0.55	32.2^{a}±1.37	1.321	0.0248
	NP %	64.7±1.45	62.2±1.40	1.557	0.2404
	IM %	7.3^{a}±0.55	5.7^{b}±0.47	0.401	0.0049
Velocity	**VSL (µm/s)**	27.0^{b}±0.63	30.2^{a}±1.02	0.303	<.0001
	VCL (µm/s)	123.6±4.27	122.6±2.77	3.418	0.8479
	VAP (µm/s)	67.0±3.98	64.7±1.39	2.811	0.5729
Movement pattern	**LIN%**	24.6±1.75	25.6±0.91	1.341	0.6024
	WOB	60.6±6.40	53.7±0.76	4.592	0.2900
	STR%	43.8±1.01	45.9±1.08	1.065	0.1596
	MAD	99.7±1.72	96.9±1.81	1.892	0.3034
	ALH (µm)	12.1±0.19	11.8±0.21	0.211	0.4138
	BCF (Hz)	3.6±0.10	3.6±0.33	0.257	0.9483

$^{a-b}$: means within a row with different superscripts differ significantly ($P<0.05$). Control: Plan water, Juice: 13.2 mg total phenols from the pomegranate Juice/rabbits. Number of samples =12.
TMOT(%): Total motility percent's, PR (%): progressive motility, NP (%): non-progressive motility, IM (%): immotile sperm, VSL (μm/sec): velocity straight line, VCL (μm/sec): velocity curvilinear, VAP (μm/sec): velocity average path, LIN (%): linearity, WOB: wobble, STR (%): straightness, MAD: mean angular displacement, ALH (μm): amplitude of lateral head displacement, BCF (Hz): beat cross frequency.

The effects of pomegranate juice administration via drinking water on serum total antioxidant capacity (TAC), malondialdehyde (MDA), testosterone concentrations and catalase (CAT) activity are summarized in Table 3.

Rabbits receiving pomegranate juice showed significantly higher TAC and lower MDA levels in serum, with no significant differences in CAT activity. Additionally, pomegranate juice-treated rabbits exhibited higher serum testosterone concentrations compared to the control group.

Table 3. Effect of oral administration of aqueous pomegranate Juice on hormones, antioxidant, and biochemical blood parameters of New Zealand Rabbit bucks (mean)

Traits	Control	Juice	SEM	P-value
TAC (nmol/ml)	1.3^{b}±0.07	1.5^{a}±0.01	0.046	0.0122
MDA (nmol/ml)	13.9^{a}±1.27	6.0^{b}±0.12	0.959	0.0021
CAT (nmol/ml)	6.5±0.87	6.6±0.80	0.841	0.9000
Testosterone (µg/ml)	3.2^{b}±0.05	3.5^{a}±0.03	0.038	0.0035

$^{a-b}$: means within a row with different superscripts differ significantly (P<0.05). Control: Plan water, Juice: 13.2 mg total phenols from the pomegranate Juice/rabbits. Number of samples =12. TAC (nmol/ml): total antioxidant capacity, MAD (nmol/ml): malonaldehyde, CAT (nmol/ml): catalase.

Discussion

Rabbits that consumed drinking water supplemented with pomegranate juice exhibited significantly reduced mount latency, the time taken by males to initiate mounting behavior toward females, compared to the control group, which received tap water. Mount latency is inversely related to sexual motivation (Yakubu and Afolayan, 2009). The shorter latency observed in the treated groups is likely linked to increased testosterone secretion, reflecting enhanced sexual motivation. This parameter serves as a key indicator of vigor, libido, and potency in male rabbits, with shorter durations indicating greater sexual drive (Ajayi and Akhigbe, 2020).

Male sexual behavior is widely acknowledged to be androgen-dependent, with circulating testosterone playing a central role in its regulation (Handelsman *et al*., 2018). The relationship between elevated testosterone levels and enhanced libido and sexual activity in male rabbits is well established (Hafez and Hafez, 2000).

Pomegranate juice is known for its rich antioxidant content (Benchagra *et al.,* 2021). Antioxidants have been shown to improve intracavernous blood flow, promote smooth muscle relaxation, and enhance erectile function in both arteriogenic erectile dysfunction (ED) and non-pathogenic conditions, while also mitigating ischemia-induced fibrosis (Azadzoi *et al*., 2005). This aligns with the findings by Katana *et al*. (2020) who reported a significant increase in intrinsic libido with pomegranate fruit extract supplementation.

The increased ejaculate volume and total sperm motility in rabbits consuming pomegranate juice compared to the control group may indicate the mitigation of oxidative stress by the antioxidant properties of pomegranate bioactive compounds.

Reactive oxygen and nitrogen species (ROS and RNS) are essential in biological systems, typically existing at low concentrations within cells. In oxygen-rich

environments, cellular components require protection against oxidative stress due to the formation of highly reactive intermediates during oxygen reduction (Fridovich, 1998). The antioxidant defense system, comprising enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), as well as vitamins (α-tocopherol and ascorbate) and carotenoids, neutralizes ROS and RNS to maintain cellular stability (Surai, 2002). However, oxidative stress can arise when ROS production surpasses the capacity of antioxidants, leading to potential cellular damage.

A significant consequence of oxidative stress is lipid peroxidation, which occurs when free radicals and ROS react with polyunsaturated fatty acids in cell membranes (Novo and Parola, 2008; Upston *et al*., 1999). Sperm cell membranes, rich in polyunsaturated fatty acids with unconjugated double bonds, are particularly vulnerable to oxidative damage. Lipid peroxidation damages approximately 60% of membrane fatty acids, resulting in decreased membrane fluidity, increased ion permeability, and impaired function of membrane-bound receptors and enzymes (Dutta *et al*., 2019). This disruption leads to a rapid decline in intracellular ATP levels, reducing sperm viability, causing axonemal damage, and increasing morphological defects in the midpiece, ultimately inhibiting spermatogenesis (Nowicka-Bauer and Nixon, 2020). As lipid peroxidation progresses, secondary products like aldehydes can modify cytoplasmic and nuclear proteins, adversely affecting mitochondrial DNA and the transcription of electron transport chain proteins (Negre-Salvayre *et al*., 2008). This disruption leads to mitochondrial dysfunction and reduced energy production (Nowicka-Bauer *et al*., 2018). The decline in ATP production further impairs sperm motility, compounded by the absence of cytoplasmic enzyme repair systems (Agarwal *et al*., 2014).

Banavath and Srinivasa (2021) found that daily exposure of male Sprague Dawley rats to mobile radiofrequency electromagnetic radiation (RF-EMR) for 90 days reduced sperm quality, including motility, while increasing abnormalities due to oxidative stress. However, pomegranate juice supplementation reversed these negative effects, suggesting it could serve as a nutritional intervention to enhance sperm quality. Additionally, aging negatively impacts fertility, with evidence showing declines in sperm motility by 3% to 12% and normal morphology by 4% to 18% over 20 years (Auger *et al*., 1995). Alshinnawy *et al.* (2020) reported that aging-induced imbalances in male sex hormones and reductions in sperm count and motility were effectively mitigated by pomegranate peel extract (250 mg/kg/day). Their findings highlight pomegranate's anti-aging effects in restoring hormonal balance and preserving testis structure, further supporting its role in improving male fertility.

Compared to the control group, rabbits given pomegranate juice exhibited significantly higher serum TAC and lower MDA levels. These findings indicate that pomegranate juice possesses potent antioxidant properties that can enhance seminal plasma composition. Pomegranate juice is particularly rich in natural antioxidants such as α-tocopherol, ascorbic acid, and a variety of polyphenols, including ellagitannins and punicalagin, which remain stable through the acidic environment of the stomach and small intestine. Furthermore, pomegranate (*Punica granatum*) contains a complex mix of polyphenolic anthocyanins, contributing to its powerful antioxidant effects (Kostka *et al.*, 2020).

Lorzadeh *et al.* (2022) explored the effects of pomegranate on oxidative stress markers, as previous studies have reported mixed outcomes regarding its antioxidant properties. Some research suggests that pomegranate can reduce malondialdehyde (MDA) levels in individuals with obesity or diabetes and increase total antioxidant capacity (TAC) (Hosseini *et al.,* 2016; Sohrab *et al.,* 2017). However, other studies found no significant impact on these or other oxidative stress indicators (Guo *et al.,* 2008; Ghavipour *et al.,* 2017). To clarify these discrepancies, Lorzadeh *et al.* (2022) conducted a systematic review and meta-analysis of randomized controlled trials (RCTs), reviewing 1692 studies and selecting 21 for detailed analysis. Their findings revealed that pomegranate consumption significantly elevated TAC levels while reducing MDA levels compared to controls.

Pomegranate juice-treated rabbits exhibited higher serum testosterone levels than controls, potentially due to pomegranate's ability to enhance blood circulation and support hormone-producing organs. Roelofs *et al*. (2017) found that pomegranate extract improved blood flow and vessel diameter during intense exercise, suggesting better oxygen and nutrient delivery.

Research on pomegranate's effect on testosterone levels remains mixed. Al-Dujaili and Smail (2012) reported a 24% increase in salivary testosterone in men and women after one to two weeks of daily pomegranate juice consumption. However, Ammar *et al.* (2020) found that pomegranate juice reduced plasma testosterone levels shortly after exercise and lowered homocysteine (Hcy) levels during recovery, with a negative correlation between Hcy and testosterone responses.

Leydig cells, located near macrophages in the testes, are vulnerable to oxidative stress from reactive oxygen species (ROS) produced during immune responses (Diemer *et al*., 2003; Riris *et al*., 2021). Disrupted antioxidant-ROS balance can impair Leydig cell function, affecting testosterone production, spermatogenesis, and fertility (Riris *et al*., 2021). Polyphenols in pomegranate, such as ellagic acid, punicalagins, gallic acid, anthocyanidins, and urolithins, exhibit strong antioxidant activity, mitigating oxidative damage and promoting testosterone synthesis (Türk *et al.,* 2008).

Conclusion

Pomegranate juice supplementation in drinking water significantly improved reproductive performance, reduced oxidative stress, and enhanced testosterone levels in male rabbits. The increase in ejaculate volume and total sperm motility, along with reduced mount latency, suggests that PJ enhances sexual behavior and semen quality. Additionally, the improved oxidative status, indicated by higher TAC and lower MDA levels, highlights the antioxidant potential of PJ in mitigating oxidative damage. The findings support the role of pomegranate as a natural supplement to enhance male fertility, offering potential applications in rabbit breeding programs. Further studies are recommended to explore the long-term effects of PJ and its mechanisms of action in other animal models.

References

Aebi, H. (1984). Catalase in vitro. Methods Enzimol. 105: 121–126.

Agarwal, A., Virk, G., Ong, C., and Du Plessis, S. S. (2014). Effect of oxidative stress on male reproduction. The world journal of men's health. 32(1): 1-17.

Ajayi, A. F., and Akhigbe, R. E. (2020). Assessment of sexual behaviour and fertility indices in male rabbits following chronic codeine use. Andrology. 8(2): 509-515.

Al-Dujaili, E., and Smail, N. (2012). Pomegranate juice intake enhances salivary testosterone levels and improves mood and well being in healthy men and women. Abstracts, 28,3-13.

Alshinnawy, A., Elsayed, W., Taha, A., Sayed, A., and Salem, A. (2020). Astragalus membranaceus and *Punica granatum* alleviate infertility and kidney dysfunction induced by aging in male rats. Turkish Journal of Biology. 44(4): 166-175.

Am-In, N., Kirkwood, R. N., Techakumphu, M., and Tantasuparuk, W. (2010). Effect of storage for 24 h at 18 °C on sperm quality and a comparison of two assays for sperm membrane lipid peroxidation. Canadian Journal of Animal Science. 90(3): 389-392.

Ammar, A., MounaTurki, Trabelsi, K., Bragazzi, N. L., Boukhris, O., Bouaziz, M., Ayadi, F., El Abed, K., Driss, T., Souissi, N., Chtourou, H., Bailey, S. J., and Hoekelmann, A. (2020). Effects of natural polyphenol-rich pomegranate juice on the acute and delayed response of Homocysteine and steroidal hormones following weightlifting exercises: a double-blind, placebo-controlled trial. Journal of the International Society of Sports Nutrition. 17: 1-13.

Attia, Y. A., Hamed, R. S., Bovera, F., Abd El, A. E. H. E., Al-Harthi, M. A., and Shahba, H. A. (2017). Semen quality, antioxidant status and reproductive performance of rabbits bucks fed milk thistle seeds and rosemary leaves. Animal reproduction science. 184: 178-186.

Auger, J., Kunstmann, J. M., Czyglik, F., and Jouannet, P. (1995). Decline in semen quality among fertile men in Paris during the past 20 years. New England Journal of Medicine. 332(5): 281-285.

Azadzoi, K. M., Schulman, R. N., Aviram, M., and Siroky, M. B. (2005). Oxidative stress in arteriogenic erectile dysfunction: prophylactic role of antioxidants. The Journal of urology. 174(1): 386-393.

Bakeer, M. R., El-Attrouny, M. M., and Abdelatty, A. M. (2021). Effect of dietary pomegranate peel (*Punica granatum* L.) and Aloe vera gel (*Aloe barbadensis* miller) supplementation on testicular antioxidant biomarkers and spermatogenesis enzymes in mature V-Line rabbit bucks. Journal of Animal Physiology and Animal Nutrition. 105(1): 175-182.

Banavath, A. N., and Srinivasa, S. N. (2021). Ameliorative effect of *Punica granat*um on sperm parameters in rats exposed to mobile radioelectromagnetic radiation. Asian Pacific Journal of Reproduction. 10(5): 225-231.

Benchagra, L., Berrougui, H., Islam, M. O., Ramchoun, M., Boulbaroud, S., Hajjaji, A., Fulop, T., Feretti, G., and Khalil, A. (2021). Antioxidant effect of moroccan pomegranate (*Punica granatum* L. sefri variety) extracts rich in punicalagin against the oxidative stress process. Foods. 10(9): 2219.

Björndahl, L., Söderlund, I., and Kvist, U. (2003). Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Human reproduction. 18(4): 813-816.

Diemer, T., Allen, J. A., Hales, K. H., and Hales, D. B. (2003). Reactive oxygen disrupts mitochondria in MA-10 tumor Leydig cells and inhibits steroidogenic acute regulatory (StAR) protein and steroidogenesis. Endocrinology. 144(7): 2882-2891.

Donnelly, E. T., McClure, N., and Lewis, S. E. (2001). Cryopreservation of human semen and prepared sperm: effects on motility parameters and DNA integrity. Fertility and sterility. 76(5): 892-900.

Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics. 11(1): 1-42.

Dutta, S., Majzoub, A., and Agarwal, A. (2019). Oxidative stress and sperm function: A systematic review on evaluation and management. Arab journal of urology. 17(2): 87-97.

Elfalleh, W., Hannachi, H., Tlili, N., Yahia, Y., Nasri, N., and Ferchichi, A. (2012). Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. Journal of Medicinal Plants Research. 6(32): 4724-4730.

Fayed, A. M., Azoz, A. A., Zedan, A. H., and Basyony, M. (2012). Effects of pomegranate peel as antioxidant supplementation on digestibility, blood biochemical and rabbit semen quality. Egy. J. Nutr. and Feeds. 15: 343-354.

Ferrara, G., Cavoski, I., Pacifico, A., Tedone, L., and Mondelli, D. (2011). Morpho-pomological and chemical characterization of pomegranate (*Punica granatum* L.) genotypes in Apulia region, Southeastern Italy. Scientia Horticulturae. 130(3): 599-606.

Fischer, U. A., Carle, R., and Kammerer, D. R. (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD–ESI/MSn. Food chemistry. 127(2): 807-821.

Fridovich, I. (1998). Oxygen toxicity: A radical explanation. Journal of Experimental Biology. 201(8): 1203-1209.

Galal, E. S. E., and Khalil, M. H. (1994). Development of rabbit industry in Egypt. *In*: Baselga M., and Marai I. F. M. (eds.). Rabbit Production in Hot Climates. Zaragoza: CIHEAM, 1994. p. 43-55 (Cahiers Options Méditerranéennes), n. 8.

Ghavipour, M., Sotoudeh, G., Tavakoli, E., Mowla, K., Hasanzadeh, J., and Mazloom, Z. (2017). Pomegranate extract alleviates disease activity and some blood biomarkers of inflammation and oxidative stress in Rheumatoid Arthritis patients. European journal of clinical nutrition. 71(1): 92-96.

Guo, C., Wei, J., Yang, J., Xu, J., Pang, W., and Jiang, Y. (2008). Pomegranate juice is potentially better than apple juice in improving antioxidant function in elderly subjects. Nutrition research. 28(2): 72-77.

Hafez, E.S.E., Hafez, B., (2000). Reproductive cycles. *In*: Hafez, E.S.E., and Hafez, B. (Eds.), Reproduction in Farm Animals, seventh ed. Lippincott Williams and Wilkins, Philadelphia, pp. 55–67.

Handelsman, D. J., Hirschberg, A. L., and Bermon, S. (2018). Circulating testosterone as the hormonal basis of sex differences in athletic performance. Endocrine reviews. 39(5): 803-829.

Hasan, M. M., El-Shal, A. S., Mackawy, A. M., Ibrahim, E. M., Abdelghany, E. M., Saeed, A. A., and El-Gendy, J. (2020). Ameliorative effect of combined low dose of Pioglitazone and omega-3 on spermatogenesis and steroidogenesis in diabetic rats. Journal of Cellular Biochemistry. 121(2): 1524-1540.

Hosseini, B., Saedisomeolia, A., Wood, L. G., Yaseri, M., and Tavasoli, S. (2016). Effects of pomegranate extract supplementation on inflammation in overweight and obese individuals: A randomized controlled clinical trial. Complementary therapies in clinical practice. 22: 44-50.

Katana, L. K., Maina, C. I., and Orenge, C. O. (2020). Aphrodisiac activity of pomegranate (*Punica granatum* L.) fruit extract on the sexual function in rats. Discovery Phytomedicine. 7(2): 65-71.

Kostka, T., Ostberg-Potthoff, J. J., Briviba, K., Matsugo, S., Winterhalter, P., and Esatbeyoglu, T. (2020). Pomegranate (*Punica granatum* L.) extract and its anthocyanin and copigment fractions. Free radical scavenging activity and influence on cellular oxidative stress. Foods. 9(11): 1617.

Lombardo, F., Sansone, A., Romanelli, F., Paoli, D., Gandini, L., and Lenzi, A. (2011). The role of antioxidant therapy in the treatment of male infertility: An overview. Asian Journal of andrology. 13(5): 690.

Lorzadeh, E., Heidary, Z., Mohammadi, M., Nadjarzadeh, A., Ramezani-Jolfaie, N., and Salehi-Abargouei, A. (2022). Does pomegranate consumption improve oxidative stress? A systematic review and meta-analysis of randomized controlled clinical trials. Clinical Nutrition ESPEN. 47: 117-127.

Negre-Salvayre, A., Coatrieux, C., Ingueneau, C., and Salvayre, R. (2008). Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. British journal of pharmacology. 153(1): 6-20.

Novo, E., and Parola, M. (2008). Redox mechanisms in hepatic chronic wound healing and fibrogenesis. Fibrogenesis and tissue repair. 1: 1-58.

Nowicka-Bauer, K., and Nixon, B. (2020). Molecular changes induced by oxidative stress that impair human sperm motility. Antioxidants. 9(2): 134.

Nowicka-Bauer, K., Lepczynski, A., Ozgo, M., Kamieniczna, M., Fraczek, M., Stanski, L., Olszewska, M., Malcher, A., Skrzypczak, W., and Kurpisz, M. K. (2018). Sperm mitochondrial dysfunction and oxidative stress as possible reasons for isolated asthenozoospermia. Journal of Physiology and Pharmacology, 69(3): 405-409.

Ohkawa, H. (1978). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 98: 351. 352.

Qasim, S. A., and Ali, A. S. (2020). Effect of pomegranate juice and fresh leaves of *Eruca vesicaria* on testosterone hormone level in blood serum of male rabbits. Systematic Reviews in Pharmacy. 11(10): 1211-1214.

Riris, A. A. I. D. C., I'tishom, R., and Khaerunnisa, S. (2021). Role of antioxidant to protect Leydig cells induced by reactive oxygen species: A literature review. Qanun Medika-Medical Journal Faculty of Medicine Muhammadiyah Surabaya. 5(1): 49-60.

Roelofs, E. J., Smith-Ryan, A. E., Trexler, E. T., Hirsch, K. R., and Mock, M. G. (2017). Effects of pomegranate extract on blood flow and vessel diameter after high-intensity exercise in young, healthy adults. European journal of sport science. 17(3): 317-325.

SAS Institute. 2009. User's Guide Version, 9.2, 2002-2009. SAS institute Inc, Cary, NC.

Singleton, V. L., and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 16(3): 144-158.

Sohrab, G., Ebrahimof, S., Sotoudeh, G., Neyestani, T. R., Angoorani, P., Hedayati, M., and Siasi, F. (2017). Effects of pomegranate juice consumption on oxidative stress in patients with type 2 diabetes: A single-blind, randomized clinical trial. International journal of food sciences and nutrition. 68(2): 249-255.

Sun, Y. Q., Xin, T. A. O., Men, X. M., Xu, Z. W., and Tian, W. A. N. G. (2017). *In vitro* and *in vivo* antioxidant activities of three major polyphenolic compounds in pomegranate peel: Ellagic acid, punicalin, and punicalagin. Journal of integrative agriculture. 16(8): 1808-1818.

Surai, P. F. (2002). Natural antioxidants in avian nutrition and reproduction (Vol. 1). Nottingham: Nottingham University Press.

Türk, G., Sönmez, M., Aydin, M., Yüce, A., Gür, S., Yüksel, M., ... and Aksoy, H. (2008). Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clinical nutrition. 27(2): 289-296.

Upston, J. M., Terentis, A. C., and Stocker, R. (1999). Tocopherol-mediated peroxidation of lipoproteins: Implications for vitamin E as a potential antiatherogenic supplement. The FASEB Journal. 13(9): 977-994.

Yakubu, M. T., and Afolayan, A. J. (2009). Effect of aqueous extract of *Bulbine natalensis* (Baker) stem on the sexual behaviour of male rats. International Journal of Andrology. 32(6): 629-636.

Zeweil, H. S., Elnagar, S., Zahran, S. M., Ahmed, M. H., and El-Gindy, Y. (2013). Pomegranate peel as a natural antioxidant boosts bucks' fertility under Egyptian summer conditions. World Rabbit Science. 21(1): 33-39.

تأثير اضافة عصير الرمان على الأداء التناسلي، وحالة الأكسدة، ومستوى هرمون التستوستيرون في ذكور الأرانب النيوزيلندية

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الملخص

تهدف هذه الدراسة لتقييم تأثير عصير الرمان (PJ) على الأداء التناسلي، وعلامات الإجهاد التأكسدي، ومستوي هرمون التستوستيرون في ذكور الأرانب النيوزيلندية. تم تقسيم 12 أرنبًا بشكل عشوائي إلى مجموعتين: مجموعة الكنترول تتناول ماء الصنبور العادي ومجموعة المعاملة تتناول 20 مل من عصير الرمان PJ يوميًا.

قيمت المعايير التناسلية، بما في ذلك زمن القفز، وجودة السائل المنوي، وحركة وحيوية الحيوانات المنوية، ومستويات هرمون التستوستيرون. بالإضافة إلى ذلك، تم قياس معلمات مضادات الأكسدة في الدم مثل مضادات الأكسدة الكلية (TAC)، والمالونديالدهيد (MDA)، ونشاط الكتاليز (CAT).

أظهرت النتائج أن تناول عصير الرمان (PJ) قلل معنوياً (P <0.05) من زمن القفز وأدي لزيادة مستوي هرمون التستوستيرون وحجم القذف وحركة الحيوانات المنوية الكلية مقارنة بمجموعة الكنترول. بينما انخفض تركيز الحيوانات المنوية لكل مليلتر (P<0.05)، ظل عدد الحيوانات المنوية الكلية دون تأثير. كشف تحليل السيرم أن الأرانب التي تناولت عصير الرمان PJ أظهرت ارتفاع في تركيز الكتاليز TAC وانخفاض تركيزالمالونديالدهيد MDA معنوياً، مما يشير إلى تحسن حالة الأكسدة. لم يلاحظ أي تغييرات معنوية في نشاط الكتاليز (CAT).

تشير هذه النتائج إلى أن تناول عصير الرمان PJ يمكن أن يعزز الأداء التناسلي ويخفف من الإجهاد التأكسدي للأرانب، ربما عن طريق دعم الدفاعات المضادة للأكسدة وإنتاج هرمون التستوستيرون. وقد يساهم المظهر الغني بمضادات الأكسدة في PJ، بما في ذلك البولي فينول والبونيكالاجين، في آثاره المفيدة على خصوبة الذكور، مما يدعم استخدامه في برامج التربية.

الكلمات المفتاحية: *البولي فينول، الحالة التأكسدية، حركة الحيوانات المنوية، جوده الحيوانات المنوية، السلوك الجنسي*