



Cryopreserved Semen of Holstein and Buffalo Bulls was Adversely Affected by High Pomegranate Juice Levels

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Abstract

THIS study investigated whether pomegranate juice (PJ) ameliorates cryopreserved semen in Holstein and buffalo bulls. Control extenders and varying concentrations of PJ (1%, 2%, 4%, 6%, and 8%) were utilized to dilute, equilibrate, and cryopreserve semen. Results demonstrated that after dilution, sperm motility and viability percentages at 6% and 8% were lower ($P < 0.05$) than for controls in Holstein bulls. However, in buffalo bulls, percentages of motile and viable sperm did not differ ($P > 0.05$) among controls and variant PJ concentrations. After equilibration in Holstein bulls, percentages of sperm motility and viability at 1% and control were higher ($P < 0.05$) than those at 8%. In buffalo bulls, percentages of sperm motility were higher at 2% ($P < 0.05$) than at 6% and 8%. In buffalo bulls, percentages of sperm motility at 2% were higher than at 8%, and percentages of sperm viability at 1% were higher ($P < 0.05$) than at 8%. In buffalo bulls, progressive sperm motility at 6% was higher than in control and 1%. In Holstein bulls at 8%, DAP, DCL, DSL, VAP, VCL, and BCF were lower ($P < 0.05$) than at 1% and control. In buffalo bulls, DAP, DCL, DSL, VAP, and VCL were lower at 1% than at 4% and 6%. In conclusion, in buffalo bulls, low doses of PJ improved sperm kinetics, but in Holstein bulls, PJ had opposite effect; nevertheless, sperm kinetics and features were negatively impacted by high concentrations.

Keywords: Bulls, semen, pomegranate juice, cryopreservation.

Introduction

In farm animals, artificial insemination (AI) is an adaptive reproductive innovation that remains superior to natural insemination in many ways, including global gene enhancement, lowering the danger of sexually transmitted infections, and managing the calving interval to close the gap between milk supply and demand [1]. The transfer of an adequate number of healthy spermatozoa to the proper reproductive tract site is one of many parameters that determine the efficacy of insemination [2]. The most effective method for maintaining male fertility is sperm cryopreservation, which is now a crucial component of assisted reproductive technology (ART) [3]. Nonetheless,

cellular cryoinjuries are crucial to the procedure. The cryoinjury results from several damage mechanisms, including intra- and extracellular ice crystals, the toxicity of cryoprotectants, osmotic stress, and cold shock [4]. The ultra-structural injury promotes oxidative and osmotic stress, alters lipid and protein structure, impairs motility and viability, damages mitochondria, and increases DNA fragmentation [5].

The ancient plant known as pomegranate (*Punica granatum L.*) is tasty and beneficial to health. One of the most popular pomegranate products is pomegranate juice (PJ), which provides an easy and fast means for utilizing beneficial components of pomegranates [6]. The health advantages of PJ are attributed to its phenolics, which include flavonoids

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(Received 01 June 2025, accepted 25 October 2025)

DOI: 10.21608/ejvs.2025.390960.2882

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and phenolic acids [7]. Diverse antioxidant groups, including vitamin C, vitamin E, ellagic acid, ellagitannins, and anthocyanins, are abundant in PJ. Furthermore, urolithins, which are products of tannin metabolism, have a strong antioxidant impact [8]. Further, previously in several oxidative stress-induced scenarios, research showed that PJ might increase antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT) and decrease oxidative indicators like malondialdehyde (MDA) and nitric oxide (NO) [9]. Moreover, trials undertaken within laboratory and in vivo showed that PJ might have anti-inflammatory attributes [10]. Antimicrobial attributes of PJ work successfully against pathogens including *Staphylococcus aureus* and *Listeria monocytogenes* [11], *Helicobacter pylori* and *Vibrio parahaemolyticus* [12], and viruses as surrogates for the human norovirus [11]. Many strategies have been tried, such as changing the extenders and freezing procedures, to lessen sperm harm. Reduced negative assets of freezing and thawing sperm have been recorded when cryoprotectant chemicals like glycerol and other ingredients like bovine serum albumin (BSA), polyvinyl alcohol (PVA), egg yolk, and milk are incorporated to extenders [13]. Bull semen cryopreservation has been accomplished using a range of extenders, including those based on milk, egg yolk, citrate, sugars, lactose, and some plant bases such as soybean lecithin nanoparticles [14]. Therefore, the current study aimed to assess the impact of various concentrations of PJ in tris-citric acid extender on the characteristics of the semen in frozen-thawed Holstein and buffalo bull's spermatozoa.

Material and Methods

Animals

The current investigation was managed in Animal Production Research Institute (APRI; Dokki, Giza, Egypt) and International Livestock Management Training Centre (ILMTC; Sakha, Kafr El Sheikh Egypt) from December 2023 to June 2024. Mature Holstein bulls ($n = 5$; 3-5 years and weighing 500 and 700 kg) and buffalo bulls ($n = 5$; 3-5 years and weighing 500 and 700 kg) provided their semen. These bulls were housed in separate stalls in semi open shed under approved animal care procedures and fed the same feed during the trial.

Semen collection

Every male was permitted to use a sterile artificial vagina to ejaculate twice a week. After being moved to the nearby lab, the semen samples were first assessed for volume (in a graduated tube), sperm motility, and live sperm percentage. For both species, only ejaculates exhibiting motility above

70%, 80% morphologically normal spermatozoa, and sperm concentration above 1×10^9 sperm/mL were incorporated into the freezing treatment. High-quality ejaculates have been combined and treated as one pooled sample of semen to get rid of the bull influence and obtain adequate semen for replications. Once the quality of the semen was determined, each pool was divided into six portions, and each was fourfold extended (v/v) at room temperature (RT) using a different type of extender.

Preparation of semen extenders

A total of 3.028 g Tris, 1.675 g Citric Acid, and 0.75 g Fructose (TCYF) constituted the basic extender. Following the dissolution of all components in 100 ml of distilled water, the basic extender was mixed with 500 µg of penicillin and 500 µg of streptomycin. Six sections made up the basic extender with egg yolk 20%, each of which was augmented with the control extender (0% P), and 1%, 2%, 4%, 6%, and 8% of PJ. Glycerol was added as a 7% cryoprotectant to the six extenders. A digital pH meter was then used to adjust the pH so that it was between 6.9 and 7.1.

Preparation of PJ

PJ was made following Abdel Moneim *et al.* [15]. Briefly, the fresh pomegranate fruits that were intact and free of visible faults were cleaned, the peel was taken off, and their red seeds were collected in a plate that had been disinfected. To get a clear, thin juice, the juice was pulled out using a professional blender using a Buchner funnel. Then, the juice was filtered and stored at -20°C until it was utilized, which could take up to two months.

Semen dilution and freezing

After the semen examination, when motility was $\geq 70\%$, collected ejaculates were separated into six equal amounts and diluted as concentrations with the six freezing extenders at room temperature (20 to 23°C). Following that, the post-dilution was evaluated. A post-equilibration assessment was carried out on the diluted semen following four hours of equilibration at 4°C . After equilibration, a high-mobility extender was enclosed in plastic straws (0.25 mL; IMV Technologies, L'Aigle, France) for cryopreservation. Subsequently, straws were kept for 45 min at 5°C . After cooling, straws were submerged in liquid nitrogen after floating horizontally in the liquid nitrogen vapor for ten minutes at a height of five centimetres.

Sperm evaluation

Following the collection of the spermatozoa, their mass activity was determined using a light microscope set to 37°C and 100x magnification. Eosin-nigrosine stain was used to calculate the

live/dead sperm ratio on a preheated slide. 200 sperm in total were counted for each semen sample. Sperm characteristics were assessed using a phase-contrast microscope (Leica, Germany) set to 37 °C in the warm stage. For this, 200 sperm were observed (10 µL) at 400x magnification. The percentage of healthy acrosomes was ascertained as previously stated after the spermatozoa had been settled with 1% glutaraldehyde in 0.165 M sodium cacodylate buffer. To evaluate the spermatozoa's plasma membrane's functional state, they underwent the Hypo-Osmotic Swelling Test (HOST). Briefly, A 100 µl aliquot of semen sample was incubated with 1 ml of 150 mOsm hypoosmotic solution at 37°C for one hour in order to perform HOST. Once the spermatozoa were incubated, 50 µl of the HOST-treated sperm were combined with an equivalent volume of 2% glutaraldehyde/0.165 M sodium cacodylate buffer (pH 7.3 at 25°C). The pooled and diluted semen samples were evaluated instantly as they were diluted (the initial evaluation), after they were thawed, and every four hours after they were chilled at 4°C (the post-equilibration phase).

Determining sperm parameter values utilizing computer-aided semen analysis (CASA)

Sperm kinematics was assayed using CASA (SpermVision™ software Minitube Hauptstraße 41. 84184 Tiefenbach, Germany) program. Distance average path (DAP, µm); Distance curve linear (DCL, µm); Distance straight line (DSL, µm); Average path velocity (VAP, µm/s); Curvilinear velocity (VCL, µm/s); Straight line velocity (VSL, µm/s); Straightness (STR, VSL/VAP); Linearity (LIN); Wobble coefficient (WOB, VAP/VCL); amplitude of lateral head displacement (ALH, µm); and beat cross frequency (BCF, Hz), and progressive sperm motility (PM%) were measured.

Statistical analysis

One-way ANOVA was used to assess the comparisons, and Turkey-Kramer's HSD was used as a post hoc test. $P < 0.05$ was considered a significant difference. JMP software version 18.0.0 (SAS Institute, Cary, NC, USA) was used for all statistical analyses. Graphpad Prism version10 software (Graphpad Software, Inc., San Diego, CA) was used to draw the figures.

Results

Impact of PJ on the post-dilution semen of Holstein and buffalo bulls

As shown in Figure 1A, the percentage of sperm motility at 6% and 8% of PJ was lower ($P < 0.05$) compared to the control in Holstein bulls. After exposing Holstein bulls to different concentrations of PJ treatment, their sperm motility percentage

remained consistent ($P > 0.05$). Nevertheless, sperm motility in buffalo bulls showed similar values ($P > 0.05$) for both the control group and various concentrations of PJ (Fig. 1B). Sperm viability in Holstein bulls showed no difference ($P > 0.05$) between 2%, 4%, 6%, and 8% of PJ. However, viability at control and 1% of PJ was higher than ($P < 0.05$) at 6% and 8% (Fig. 1A). Sperm viability in buffalo bulls was comparable between PJ group and control group (Fig. 1B) with no difference ($P > 0.05$).

The abnormal sperm morphology percent was similar ($P > 0.05$) at 2%, 4%, 6%, and 8% of PJ, but the abnormal sperm morphology percentage at control and 1% of PJ was lower than ($P < 0.05$) it was for 4%, 6%, and 8% (Fig. 1A) in Holstein bulls. In buffalo bulls, the abnormal sperm morphology percentage at 1% of PJ was lower ($P < 0.05$) than it was at 8% (Fig. 1B). The HOST and acrosome integrity percent of the control and PJ concentration treatments ($P > 0.05$) did not differ in Holstein (Fig. 1) and buffalo bulls (Fig. 1B).

Impact of PJ on Holstein and buffalo bulls' semen post-equilibration

In Holstein bulls, there was an increase ($P < 0.05$) in both sperm motility and viability percentages at 1% of PJ and control compared to 8% (Fig. 2A). In buffalo bulls, the sperm motility was higher at 2% ($P < 0.05$) than at 6% and 8%, while the sperm viability showed similar values ($P > 0.05$) (Fig. 2B). The percentage of abnormal sperm morphology in Holstein bulls was lower ($P < 0.05$) at 1% of PJ and control groups compared to 2%, 4%, 6%, and 8% (Fig. 2A). The percentage of abnormal sperm morphology was lower ($P < 0.05$) at 1% of PJ compared to 6% in buffalo bulls (Fig. 2B). Furthermore, there were no differences ($P > 0.05$) in the HOST and acrosome integrity values between the treatment groups and the control groups for both Holstein (Fig. 2) and buffalo bulls (Fig. 2B).

Impact of PJ on Holstein and buffalo bulls' semen post-thawing

No differences ($P > 0.05$) were observed in the percentage of sperm motility, sperm viability, or acrosome integrity among the treatment groups or control in Holstein bulls (Fig. 3A). In buffalo bulls, the percentage of sperm motility was greater at 2% compared to 8% PJ, but the percentage of sperm viability at 1% PJ was higher than at 8% ($P < 0.05$) (Fig. 3B).

In Holstein bulls, the percentage of abnormal sperm morphology was higher ($P < 0.05$) at 8% of PJ compared to the control and 1% (Fig. 3A), and the HOST was lower ($P < 0.05$) compared to control at 8% of PJ (Fig. 3). The percentage of abnormal sperm morphology was higher ($P < 0.05$) at 6% and 8% of

PJ in buffalo bulls compared to 1% (Fig. 3B) ($P < 0.05$). Treatment groups and control groups exhibited comparable values for both HOST and acrosome integrity (Fig. 3B).

Kinetic parameters of post-thawed Holstein and buffalo bulls' semen treated with PJ

At 6% of PJ, the PM increased, whereas they had comparable values ($P > 0.05$); however, the mean values of PM were similar in various treatment groups and control in Holstein bulls (Fig. 4A). In buffalo bulls, the PM at 6% of PJ was higher than control and 1% (Fig. 4B).

Furthermore, DAP, DCL, DSL, VAP, and VCL were all lower ($P < 0.05$) at 8% in Holstein bulls than at 1% and control (Fig. 4A). The VSL, STR, LIN, WOB, and ALH values at 8% of PJ and control did not change ($P > 0.05$) (Fig. 5A); however, the BCF values were lower ($P < 0.05$) than those at 1% and 2% of PJ or control. DAP, DCL, DSL, VAP, and VCL were all decreased in buffalo bulls at 1% compared to 4% and 6% (Fig. 4B). Compared to 4%, 6%, and 8%, VSL was lower at 1% (Fig. 5B). Compared to 1%, 2%, and 8%, STR and LIN were higher at 4% (Fig. 5B). Compared to those at 2%, 4%, 6%, and 8%, ALH at 1% was lower (Fig. 5B).

Discussion

The development of intracellular ice crystals is a significant challenge during cryopreservation. Spermatozoa undergo structural and molecular changes due to cryodamage brought on by cryopreservation [16,17]. While storage in liquid nitrogen and the addition of cryoprotectants are included in all cryopreservation methods, slow freezing, rapid freezing, and ultrarapid freezing have some distinctions between the protocols. The primary distinction is the freezing and thawing rate, followed by the concentration and application of a cryoprotectant [18]. This study's objective was to look into the quality of cryopreserved Holstein and buffalo bulls' semen (motility, viability, morphology, plasma membrane integrity, and vigor and progression kinetics) after dilution, equilibration, and post-thawing deplying PJ.

Our results showed that, following dilution, Holstein bulls' sperm motility and viability percentages at 6% and 8% were significantly less than the control groups. After equilibration, there was a significant increase in both sperm motility and viability percentages at 1% of PJ and control compared to 8%. After thawing, no significant differences were observed in the percentage of sperm motility, sperm viability, or acrosome integrity among the treatment and control groups. The percentage of abnormal sperm morphology following dilution and thawing was significantly lower at 1%

of PJ than it was at 8%; however, after equilibration, the percentage of abnormal sperm morphology was significantly lower at 1% of PJ compared to 6%.

El-Sheshtawy *et al.* [19] investigated how various levels of PJ (10%, 20%, 30%, 40%, and 50%) added to the cattle bull semen extender affected the quality of the semen after thawing. The authors demonstrated that using 10% PJ in chilled cattle semen extended over 10 days resulted in the highest significant motility percentage. The least favorable outcomes were achieved with the utilization of 40% and 50% PJ. For frozen-thawed semen, adding 10% and 20% PJ to the extender resulted in higher post-thaw motility compared to the control group. The live percentage count significantly increased when 10% PJ was added, surpassing that of the control group. There were no noticeable variations observed in sperm membrane integrity and total sperm abnormalities among the groups. Seasonal and species differences may be the cause of the variation in PJ concentration between the current study and the aforementioned studies.

Our findings indicated that following dilution in buffalo bulls, The percentage of motile and viable sperm did not significantly change between the control and variant PJ. But as control, after equilibration, sperm motility was significantly higher at 2% than at 6% and 8%, while sperm viability showed similar values. After thawing, the percentage of sperm motility was greater at 2% compared to 8% PJ. The percentage of abnormal sperm morphology after dilution and after thawing was significantly lower at 1% PJ than it was at 8%. Following equilibration, the percentage of abnormal sperm morphology was significantly lower at 1% PJ compared to 6%.

According to Javed *et al.* [20], the field fertility, motion characteristics, and semen parameters of frozen-thawed buffalo bull spermatozoa were all affected by varying PJ concentrations (2.5%, 5%, 7.5%, and 10%) in tris-citric acid extender. In comparison to the control group, Javed *et al.* [20] found that adding 10% PJ to the extender greatly enhanced post-thaw sperm morphology and motilities (CASA total motility, progressive motility as well as VAP, VSL, VCL, STR, DAP, and DSL). Compared to the control group, the extender with 10% PJ had significantly higher plasma membrane, acrosome membrane, and DNA integrity. When comparing the extender with 10% PJ to the control, the field fertility rate was noticeably higher.

Adding different doses of pomegranate peel methanolic extract (PPME; 1.6, 2.0, 2.4, and 2.8 mg/5 ml Tris-citric acid glucose-egg yolk extender) to diluted rabbit semen, El Seadawy *et al.* [21] evaluated the antioxidant enzyme and lipid

peroxidation activities during chilled storage. In comparison to the control, the authors found that adding 1.6–2.8 mg of PPME to the chilled seminal plasma improved glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) concentrations while lowering MDA and H₂O₂ concentrations. However, at dosages of 2.4 and 2.8 mg, the most efficient PPME enhancements maintained the highest levels of SOD, CAT, and GSH.

Perumal and Rajkhowa [22] investigated the potential function of PJ (6, 8, and 10 mL of PJ/100 mL of diluent were added to semen that had been diluted with a 1:2 TEYC extender.) as a strong antioxidant in preventing the negative effects of lipid peroxidation that naturally happened when Mithun bull semen was stored in vitro at 5°C for 0, 6, 12, 24, and 30 hours. Adding PJ to the diluent caused a notable reduction in the levels of acrosomal abnormalities, dead spermatozoa, and abnormal spermatozoa during various storage time intervals. Moreover, PJ treatments at 6 and 10 ml showed less effectiveness compared to the PJ 8 ml treatment in terms of these features. It was determined that the potential benefits of PJ on sperm parameters involve boosting antioxidant enzyme function and preventing cholesterol and phospholipids from leaving the cell membrane during storage [22]. Dkhil et al. [23] showed that PJ has strong antioxidant properties by lowering the production of nitric oxide and lipid peroxidation in rat testis tissues. Both enzymatic and non-enzymatic antioxidant defense components, including GSH, CAT, SOD, GR, GST, and GPx, were included in those activities. Additionally, PJ may have physiologically active components that might combat oxidative stress, which could explain why it has been used traditionally to treat environmental pollutants [23].

The cause of the increase in sperm quality seen in this study may be that through the antioxidant capacity of PJ, spermatozoa themselves limit excessive formation of free radicals. It was determined that PJ supplementation may have protective effects by increasing the concentration of antioxidant enzymes and blocking the efflux of phospholipids and cholesterol from cell membranes. Therefore, it might improve farm animal fertility and safeguard spermatozoa during preservation [22].

Moreover, the antioxidants (polyphenols, vitamin C, E, anthocyanins, punicalagin, ellagic and gallic acid) in the PJ may be the cause of the improvement in post-thaw sperm motility metrics [24]. It has been shown that the spermatozoa's improved antioxidant enzyme profile and ROS scavenging activity following cryopreservation may be the cause of the PJ's antioxidant benefits. The primary cause of spermatogenic dysfunctions, oxidative stress and

peroxidative damage, are prevented by this antioxidant defence mechanism [25]. ROS weaken the germ layer, decrease spermatogenic cell density, decrease sperm motility, and increase the formation of aberrant sperm cells in the absence of this antioxidant defence system [26].

Each sperm is classified as possessing either progressive or non-progressive motility based on its motility characteristics [27]. Only the proportion of progressively motile sperm has been shown to be related to conception rates in terms of fertility. According to Cabrillana et al. [28], pregnancy rate is typically stated as a percentage of sperm motility, which is indicative of sperm fertility and demonstrates appropriate spermatogenesis and sperm maturation during epididymal transport. According to Nagy et al. [29], CASA enables a highly repeatable, precise, and objective evaluation of several sperm motility properties. In our study, the mean PM values in the Holstein bulls' treatment groups and control were non-significant, although PM increased at 6% of PJ, while their values remained similar. However, in buffalo bulls, the PM at 6% of PJ was significantly higher than both control and 1%.

In Holstein bulls, the kinetic parameters of PR sperm (DAP, DCL, DSL, VAP, and VCL) were significantly lower at 8% than at 1% and control. The VSL, STR, LIN, WOB, and ALH values at 8% of PJ and control did not change; however, the BCF values were significantly lower than those at 1% and 2% of PJ or control. In buffalo bulls, the kinetics (DAP, DCL, DSL, VAP, and VCL) at 4% and 6% were significantly higher than at 1%. The VSL at 4%, 6%, and 8% was significantly lower than at 1%. Compared to those at 2%, 4%, 6%, and 8%, ALH at 1% was significantly lower. The assessment of sperm kinematic characteristics in relation to fertilizing potential in bulls revealed a correlation between the success of pregnancy and all post-thaw values of VCL, VSL, and VAP for artificial insemination [30]. Nagy et al. [26] concluded that VCL, VSL, and VAP are the three kinematic parameters that have the strongest connection with fertility; among these, the most practical semen motility trait with clinical significance for predicting post-thawed bull semen fertility is VAP. In cryopreserved bull semen, substantial positive correlations were found between pregnancy rates and the kinetic parameters of VCL, VSL, BCF, and ALH [32]. Significant relationships between bull spermatozoa kinetic characteristics and in vivo fertility have been demonstrated by other researchers [29]. In line with the current research, velocity parameters associated with progressive motility suggest that spermatozoa with a straight linear route and forward motility may go farther in less time [31].

Conclusion

The frequently utilized semen in artificial insemination necessitates the analysis of several seminal characteristics, including viability, forward progressive motility, and acrosomal membrane integrity. PJ supplementation in these measures in the current investigation revealed a non-significant rise in the semen of Holstein and buffalo bulls between the treatment and control groups. Low levels of PJ in buffalo bulls enhance sperm kinetics as well as enhance sperm characteristic such as PM, whereas in Holstein bulls, PJ negatively impacts sperm kinetics. The high concentrations adversely affected the cryopreserved semen of Holstein and buffalo bulls.

Funding statement

This work was supported through the Annual Funding track by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [KFU253436].

Declaration of Conflict of Interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Ethical of approval

All experimental procedures used in the current study were approved by the guidelines of The Institutional Animal Care and Use Committee (ARC-IACUC) protocol number (ARC - APRI 57-23) Agricultural Research Centre, Egypt.

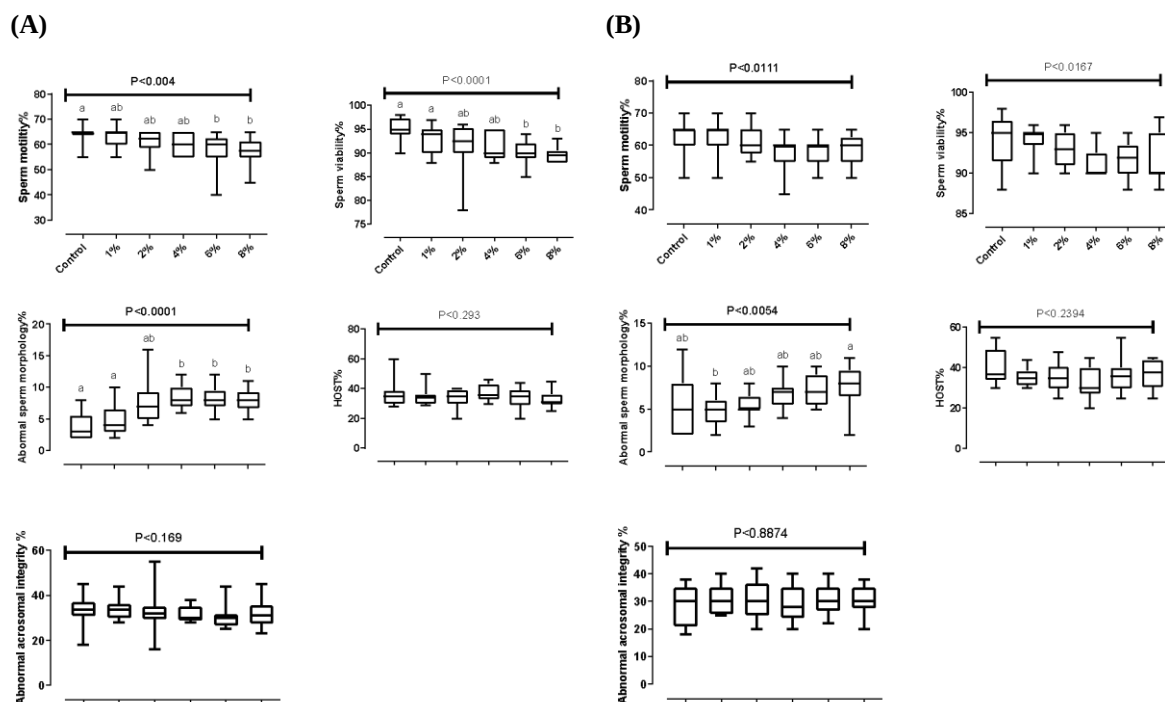


Fig. 1. Box plots show sperm characteristics of Holstein (A) and buffalo bulls (B) following dilution with pomegranate juice (PJ) (1%, 2%, 4%, 6%, or 8%; a semen sample without PJ was used as a control). ^{ab} letters indicate a significant difference between the experimental groups ($P < 0.05$).

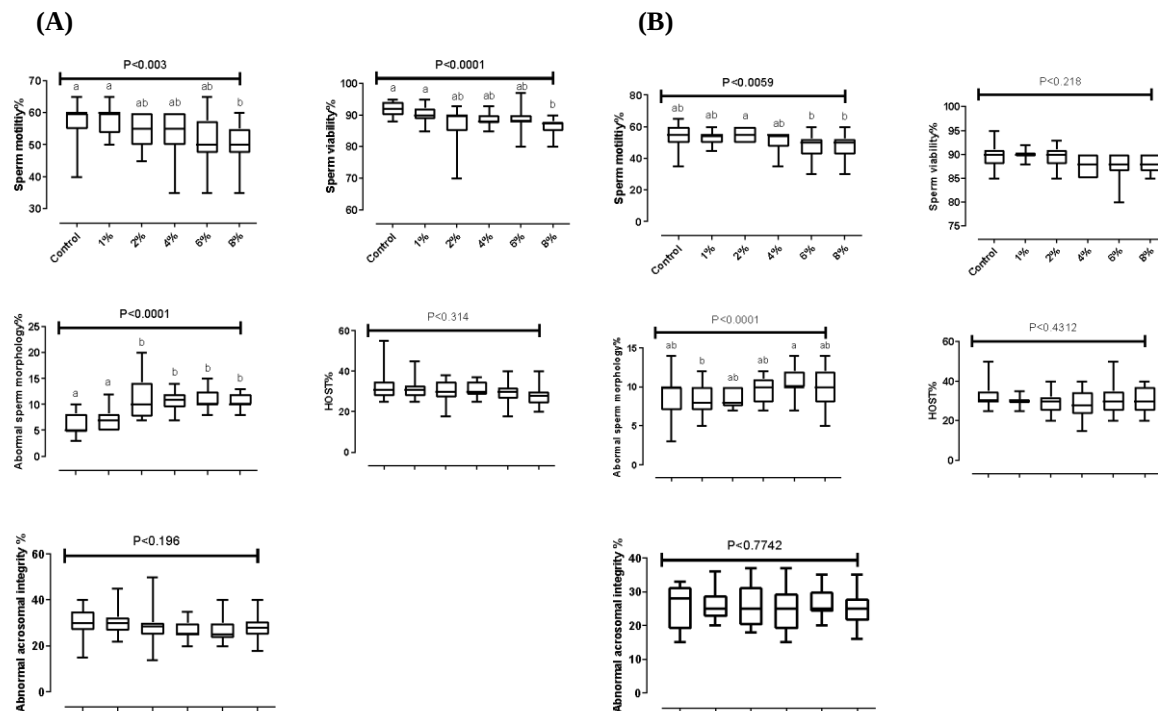


Fig. 2. Box plots show sperm characteristics of Holstein (A) and buffalo bulls (B) following equilibration with pomegranate juice (PJ) (1%, 2%, 4%, 6%, or 8%; a semen sample without PJ was used as a control). ^{ab} letters indicate a significant difference between experimental groups ($P < 0.05$).

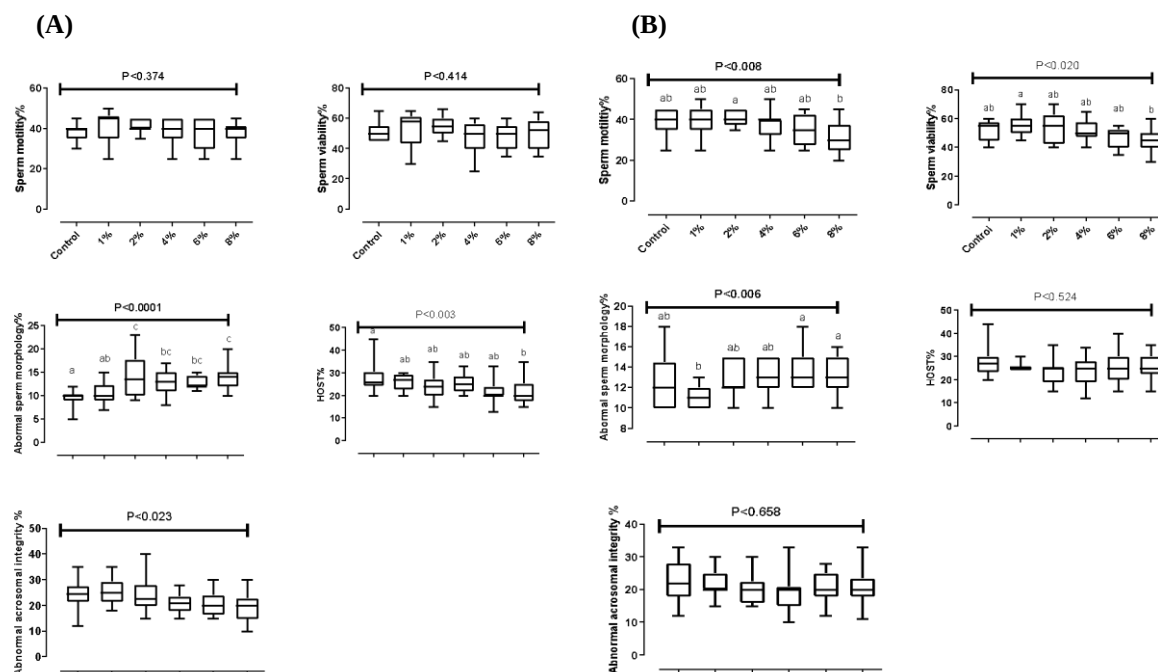


Fig. 3. Box plots show sperm characteristics of Holstein (A) and buffalo bulls (B) following thawing with pomegranate juice (PJ) (1%, 2%, 4%, 6%, or 8%; a semen sample without PJ was used as a control). ^{ac} letters indicate a significant difference between the experimental groups ($P < 0.05$).

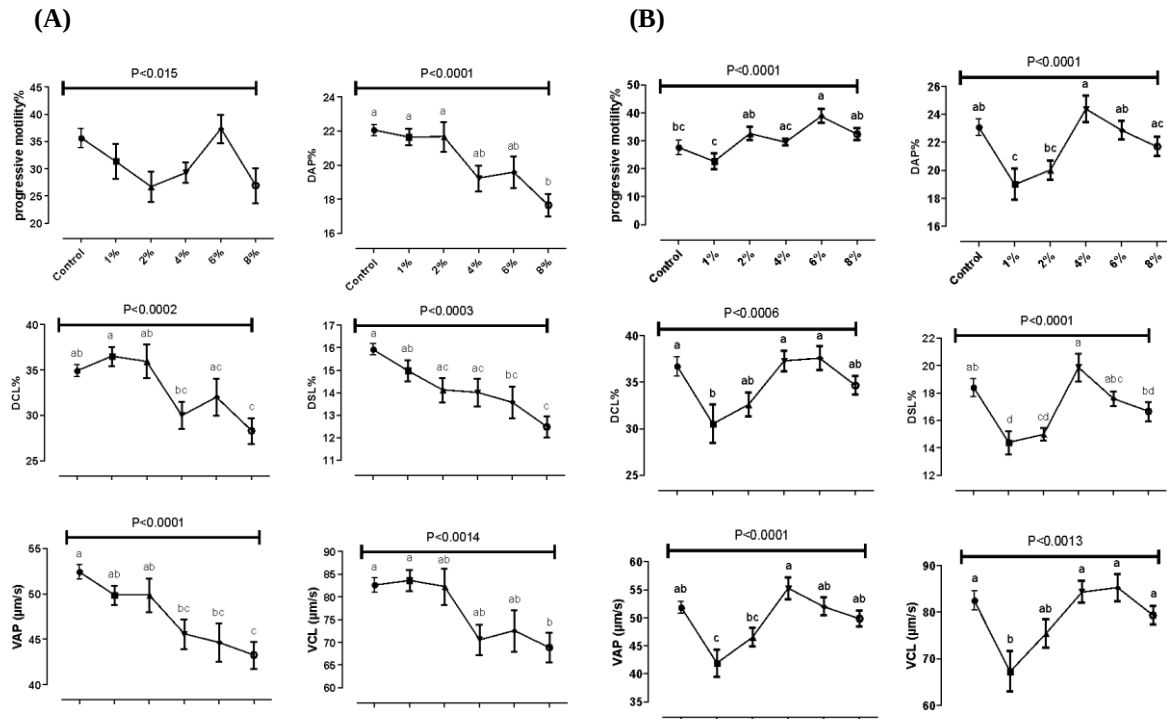


Fig. 4. Vigor and progression parameters of parameters of Holstein (A) and buffalo bulls (B) following thawing with pomegranate juice (PJ) (1%, 2%, 4%, 6%, or 8%; a semen sample without PJ was used as a control). Distance average path (DAP, μm); Distance curve linear (DCL, μm); Distance straight line (DSL, μm); Average path velocity (VAP, $\mu\text{m/s}$); Curvilinear velocity (VCL, $\mu\text{m/s}$). ^{ac} letters indicate a significant difference between the experimental groups ($P < 0.05$).

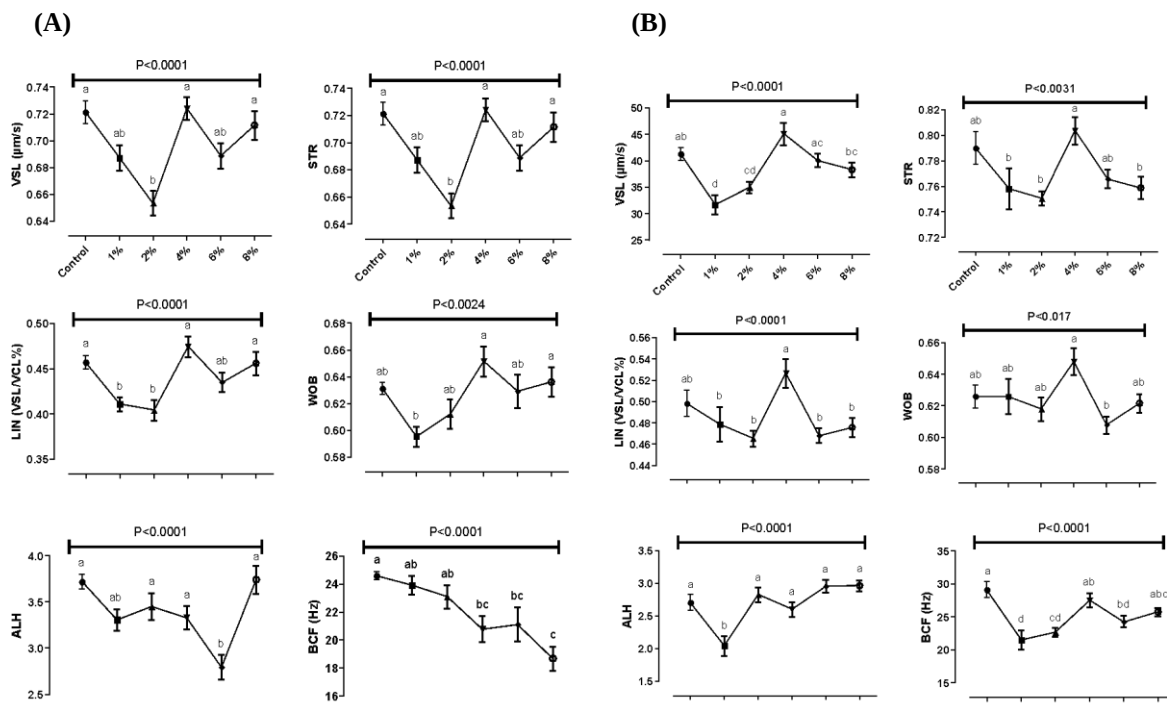


Fig. 5. Vigor and progression parameters of parameters of Holstein (A) and buffalo bulls (B) following thawing with pomegranate juice (PJ) (1%, 2%, 4%, 6%, or 8%; a semen sample without PJ was used as a control). Straight line velocity (VSL, $\mu\text{m/s}$); Straightness (STR, VSL/VAP); Linearity (LIN); Wobble coefficient (WOB, VAP/VCL); Amplitude of lateral head displacement (ALH, μm); Beat cross frequency (BCF, Hz). ^{ac} letters indicate a significant difference between the experimental groups ($P < 0.05$).

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تأثر السائل المنوي المحفوظ بالتجميد لثيران الهولشتاين والجاموس سلبيًا بارتفاع مستويات عصير الرمان

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الملخص

بحثت هذه الدراسة فيما إذا كان عصير الرمان (PJ) يخفف من السائل المنوي المحفوظ بالتبريد في ثيران هولشتاين والجاموس. تم استخدام موسعات التحكم وتركيزات مختلفة من PJ (1%، 2%، 4%، 6%، 8%) لتخفيف السائل المنوي وتوازنه وحفظه بالتبريد. أظهرت النتائج أنه بعد التخفيف، كانت نسبة حركة الحيوانات المنوية وحيويتها عند 6% و 8% أقل ($P < 0.05$) مقارنة بالضوابط في ثيران الهولشتاين. ومع ذلك، في ثيران الجاموس، لم تختلف النسب المئوية للحيوانات المنوية المتحركة والقابلة للحياة ($P > 0.05$) بين الضوابط وتركيزات PJ المتغيرة. بعد الموازنة في ثيران هولشتاين، كانت النسب المئوية لحركة الحيوانات المنوية وقدرتها على الحياة عند التحكم 1% أعلى ($P < 0.05$) من تلك عند 8%. في الثيران الجاموسية، كانت نسبة حركة الحيوانات المنوية أعلى عند 2% ($P < 0.05$) مقارنة بـ 6% و 8%؛ ومع ذلك، أظهرت النسبة المئوية لبقاء الحيوانات المنوية قيمًا مماثلة ($P > 0.05$). بعد ذوبان الجليد في ثيران هولشتاين، لم تكن هناك اختلافات ($P > 0.05$) في حركة الحيوانات المنوية وقدرتها على البقاء ونسب سلامة الأكروسوم بين مجموعات العلاج أو السيطرة. عند 6% من PJ، زاد PM؛ ومع ذلك، كانت القيم المتوسطة PM مماثلة ($P > 0.05$). في ثيران الجاموس، كانت نسبة حركة الحيوانات المنوية عند 2% أعلى من 8%، وكانت النسبة المئوية لحيوية الحيوانات المنوية عند 1% أعلى ($P < 0.05$) مقارنة بـ 8%. في الثيران الجاموسية، كان PM عند 6% أعلى منه في السيطرة و 1%. في ثيران هولشتاين عند 8%، كانت DAP و DCL و DSL و VAP و VCL و BCF أقل ($P < 0.05$) مقارنة بـ 1% والتحكم. في الثيران الجاموسية، كانت DAP و DCL و DSL و VAP و VCL أقل بنسبة 1% مقارنة بـ 4% و 6%. في الختام، أدى علاج PJ إلى زيادة حركة الحيوانات المنوية وقدرتها على البقاء ($P > 0.05$). في ثيران الجاموس، أدت الجرعات المنخفضة من PJ إلى تحسين حركية الحيوانات المنوية، ولكن في ثيران هولشتاين، كان لـ PJ تأثير معاكس؛ ومع ذلك، تأثرت حركية الحيوانات المنوية وخصائصها سلبيًا بالتركيزات العالية.

الكلمات الدالة: جودة الثيران، السائل المنوي، عصير الرمان، الحفظ بالتبريد.