Egypt. J. Vet. Sci. **Vol. 56,** No. 3, pp. 617-626 (2025)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Effect of Adding Autologous Platelet-rich Plasma to the Freezing Extender on the Post-thaw Quality and *In vitro* Fertility of Buffalo (*Bubalus Bubalis*) Spermatozoa

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Abstract

THIS STUDY investigates the consequence of adding platelet-rich plasma (PRP) on the post-thawed quality and *in vitro* fertility of buffalo bull semen. Fifty-four ejaculates from nine buffalo bulls were collected, and every ejaculate was then dispensed into 5 equal parts, diluted with Optixcell extender supplemented with five [0, 5, 10, 15, and 20] proportions of PRP followed by freezing using the usual protocol. Post-thaw sperm motility, sperm viability, the integrity (%) of the plasma membrane, acrosome, DNA, and mitochondrial membrane potential (%) were evaluated. Total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) content as well as *in vitro* fertilization (IVF) were also assessed. The obtained results revealed that the 15 % PRP-supplemented extender improved sperm viability, progressive motility, and integrity of the plasma membrane. All 5 %, 10 %, and 15 % PRP treatments greatly reduced ($P < 0.05$) MDA and increased GPx and SOD activities. The cleavage rate was greatly increased ($P < 0.05$) following IVF with 15 % PRP-supplemented spermatozoa. In conclusion, the incorporation of 15 % PRP into the freezing extender is recommended to ameliorate freezing-thawing damage on sperm cell membranes and increase the *in vitro* fertility of buffalo bull spermatozoa.

Keywords: Buffalo, Frozen-thawed, Mitochondrial membrane, *In vitro* fertilization, PRP.

Introduction

Artificial insemination performance is based on understanding the mechanisms and constraints of semen cryopreservation. Both structural and functional sperm attributes have been linked to the process of cryopreservation, including

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(Received 06/01/2024, accepted 29/04/2024)
DOI: 10.21608/EJVS.2024.254249.1712

decreased viability and motility, damage to the plasma membrane, diminished mitochondrial membrane integrity and compromised stability of the genome [1]. Cryodamage of sperm is provoked primarily by cold shock, osmotic alterations, the formation of intracellular ice crystals, and oxidative stress [2]. Considering this, several cryoprotectants, such as fatty acids, antioxidants, peptide compounds, nanomaterial particles, animal serum, plant essential oils, and bioactive analogues, have been suggested and developed [3], also platelet-rich plasma (PRP), was incorporated into semen extender to improve frozen-thawed human [4] and bovine [5] sperm quality.

PRP is being utilized in different medical fields and bioactivity, whereas the therapeutic impact of PRP is mostly derived from the different cytokines it contains, for example, nerve growth factor (NGF), transforming growth factors β, epidermal growth factor, endothelial growth factor (EGF), peptide hormones, fibroblast growth factor (FGF), and insulin-like growth factor 1 (IGF-1) [6]. It is suggested that PRP has a defensive effect on sperm cell membranes due to its antioxidant properties. Additionally, it improves sperm motility in buffalo bulls by increasing intracellular calcium levels through ionic transference [7].

Platelet-dense granules are components of PRP, which contain histamine, adenosine triphosphate, zinc and calcium ions, superoxide dismutase (SOD), and serotonin, which are crucial for regulating cell and tissue homeostasis [8]. However, PRP components have improved sperm quality, function, and antioxidant activity in human [4, 9] and bovine [5] semen and a few publications are available on its effect on buffalo semen. Therefore, the current study investigates the possible impacts of incorporating various concentrations of autologous PRP into the extender on post-thaw semen properties, antioxidant enzymatic activity, and *in vitro* fertility of buffalo bull spermatozoa.

Material and Methods

Ethical statement

The Ethical Committee of the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt authorized all animal experiments (30–08–20–KSU). All animal experiments were conducted according to the ARRIVE guidelines (https://arriveguidelines.org).

Experimental animals

Nine buffalo (*Bubalus bubalis*) bulls aged 3 – 6 years were used in this trial to collect semen samples during a regular sperm collection procedure. Six ejaculates were collected by an artificial vagina from each buffalo bull (in total 54 ejaculates were collected). This study was carried out at the International Livestock Management Training Center, Sakha, Animal Production Research Institute, Agricultural Research Center, Kafrelsheikh, Egypt (and longitude 30° 56’ E and latitude 31° 06’ N) from September 2020 to March 2021.

Preparation of platelet-rich plasma

PRP was extracted from fresh blood collected from the jugular vein of the same nine clinically healthy and fertile buffalo bulls on the day of semen collection under complete aseptic conditions. Blood was drawn into 10 mL capacity sterile sodium citrate tubes (BD Vacutainer®, Becton Drive, Franklin Lakes, NJ, USA) [10]. The collected blood samples were centrifuged for 5 min at 300 *g* to precipitate red blood cells, and the recovered plasma was centrifuged again for 17 min at 700 *g* to collect PRP [11].

Insulin-like growth factor 1 concentration in the collected PRP

The IGF-1 concentration in the collected PRP was measured according to Echternkamp et al. 1990 [12] by using an immuno-radiometric test kit (Immunotech SAS, Marseille, France) following an acid-ethanol extraction (4 °C for 16 h), with an intra-assay coefficient of variation of 9.2 %.

Semen cryopreservation

Immediately, after semen collection, the collected ejaculates were submitted to a regular physical examination [13], and ejaculates with primary sperm motility of ≥ 70 % were involved in the cryopreservation procedures. A density spectrophotometer (SDM-5, Minitub, GmbH, Germany) was used to calculate sperm cell concentration (1 x 10^6/mL). Each bull’s ejaculate was diluted to 80 x 10^6/mL with Optixcell® (LP, IMV, L’Aigla, France) extender [14] and then dispensed into five aliquots. The collected PRP was added to the diluted semen in four different proportions: 5 %, 10 %, 15 %, and 20 %, with the fifth aliquot serving as the control (0 %). Afterwards, the diluted semen samples supplemented with five proportions of PRP were loaded in 0.25 mL mini-straws (IMV, France), and then cryopreserved with standard procedures after equilibrating for 4 h at 4 °C [15].

Semen evaluation

Following a minimum of two weeks of frozen storage, frozen straws (4 straws/PRP concentration/animal/4 replicates) were thawed for exactly 1 min at 39 °C and evacuated in a 1.5 mL microcentrifuge tube [16] to be evaluated for the following post-thaw sperm characteristics:

Sperm motility and viability (%)

A computer-aided sperm motion analyzer system (CASA; Hamilton–Thorne Biosciences, Beverly, MA, USA) was utilized for total (%) and progressive (%) motilities evaluation [17]. Briefly, 10 µL of sperm was placed within a pre-warmed (37 °C) clean Makler chamber and examined with a 100x objective. The proportion of viable spermatozoa was estimated with an eosin-nigrosin stain [18]. Two hundred sperm cells have been evaluated with an oil immersion lens (1000x), where unstained sperm cells were expressed as viability %.

Plasma membrane integrity (%)

The hypo-osmotic swelling test was used to assess the functional plasma membrane integrity [19]. In brief, 10 µL of sperm was dispersed in 100 µL of hypo-osmotic solution (150 mOsm /kg) and incubated for 30 – 60 min at 37 °C. Coiling and bending of sperm tails were counted by spotting 2 µL of incubated sperm on a pre-warmed slide and examined under a 400x objective.

Acrosomal membrane integrity (%)

A fluorescein isothiocyanate-conjugated peanut agglutinin (FITC-PNA) stained semen smear was used to detect the integrity of the acrosomal membrane [20]. Briefly, 10 µL of frozen-thawed semen was fixed in 4 % paraformaldehyde for 30 min. Fixed spermatozoa (5 µL) were spread, dried, and mounted for 5 min with 1 % (v/v) Triton X-100, and after that, dried before FITC-PNA (20 µg/mL in PBS solution) staining in darkness for 30 min. Each stained smear was washed with PBS, then dried, and examined under a phase-contrast microscope equipped with fluorescence illumination (Olympus, Japan). Sperm cells of intact-acrosome have a well-defined green fluorescence colour in the acrosomal cap, while acrosome of spermatozoa with faint fluorescence colour or even no colour were categorized as damaged.

Sperm DNA integrity (%)

Sperm DNA integrity (%) was determined as described by Martins et al. [21]. Briefly, three slides were performed for each group by distributing 20 µL of semen throughout the slide. To evaluate chromatin stability, prepared slides were dried then put in Tampon buffer (15 mmol/L $Na_2HPO_4.7H_2O$ and 80 mmol/L citric acid, pH 2.5) for 10 min at 75 °C, then stained with acridine orange (0.2 mg/mL). For each sample, 200 nuclei were randomly chosen and examined under an epifluorescence microscope where nuclei exhibiting green fluorescence have intact DNA whereas; those exhibiting red fluorescence have damaged DNA.

Mitochondrial membrane potential (%)

Mitochondrial membrane potential (MMP; %) was assessed by using JC–1, 0.15 mmoL in DMSO (5, 5', 6, 6'-tetrachloro-1, 1', 3, 3'-tetraethylbenzimidazolylcarbocyanine iodide) as previously detailed by Francis et al. [22]. Briefly, incubation of 10 µL JC–1 with 100 µL semen at 37 °C for 10 min in a dark room, then, 10 µL of this solution was visualized under an epifluorescence microscope. For each sample, the mitochondrial area of 200 spermatozoa was evaluated for relatively greater MMP (orange colour) and lesser MMP (green colour).

Antioxidant activity and lipid peroxidation

Frozen-thawed (3 straws/PRP proportion/animal/5 replicates) straws were centrifuged for 10 min at 1000 *g*, and the obtained supernatants were examined for the following biochemical analyses:

Total antioxidant capacity

Total antioxidant capacity (TAC) was evaluated using the Cayman antioxidant test (Michigan, USA) kit. Briefly, in each well on the plate 10 µL of sample or standard in duplicate +150 µL of chromogen + 10 µL of metmyoglobin were added. To initiate the reaction, 40 µL of H_2O_2 was added. After incubating the plate for 5 min, the absorbance of the samples was evaluated at 750 nm using a plate reader, and the TAC (Mm) was determined according to Lone et al. [23].

Glutathione peroxidase activity

The Cayman glutathione peroxidase (GPx) test kits were used to measure GPx activity. Briefly, the reaction was initiated by mixing a 50 µL co-substrate mixture with 100 µL of assay buffer, and 20 µL standards/samples within each well on the plate, followed by 20 µL of cumene hydroperoxide. The absorbance was recorded at 340 nm every minute with a plate reader to get a minimum of five readings. The GPx standards were used to plot the standard curve, and the GPx (nmol/min/mL) for samples was determined [19].

Superoxide dismutase activity

Superoxide dismutase test kits were used to measure SOD activity. Briefly, in every well, 200 µL of the diluted radical detector plus 10 µL of standards/samples were mixed with 20 µL of diluted xanthine oxidase, and then the plate was incubated at room temperature with gentle shaking for 20 min. Afterwards, the standard curves were designated using SOD standards, and SOD activity (U/mL) for samples were determined [15].

Malondialdehyde content

Lipid peroxidation was determined by measuring the content of malondialdehyde (MDA) using the Cayman TABARS assay kits. In brief, 10 µL of samples/standards + 400 µL colour reagent + 10 µL of sodium dodecyl sulfate were placed into a clean test tube, then incubated in a boiling water bath for 60 min, then, the suspensions were kept on crushed ice for 10 min followed by centrifugation for 10 min at 1600 *g*. An aliquot (150 µL) of supernatant was put onto the colorimetric plate and the absorbance was determined at 535 nm. The standard curve was drawn using MDA standards, and MDA content (µM/mL) for each sample was determined [15].

In vitro fertilization rate (%)

A total of 98 ovaries were collected immediately after slaughter and transported in a thermo flask containing warm (37 °C) normal saline (0.9 %). The methods for recovery of cumulus-oocyte complex (COC), *in vitro* maturation (IVM), and *in vitro* fertilization (IVF) were carried out in tissue culture medium (TCM) 199 with fetal calf serum (FCS) as described by Pavasuthipaisit et al. [24]. Briefly, COCs were aspirated from 2 to 6 mm ovarian follicles. Then, IVM was performed on 150 good-quality cumulus-oocyte complexes (oocytes with homogeneous ooplasm enclosed within compacted various cumulus cell layers) in groups of 3، in 50 µL TCM-199 supplemented with 0.2 mm pyruvate, 10 % FCS, and 5 mg/mL FSH for 22 h at 39 °C under 5 % CO_2 in the air with maximum humidity.

The *in vitro* matured oocytes were fertilized with 1 x 10^6 sperm cells capacitated with 10 mg/mL heparin. Following IVF the *in vitro*-produced zygotes were visualized under a stereomicroscope to evaluate the number of cleaved oocytes [25]. The IVF capability of treatments was evaluated by estimating the cleavage rate and applying the equation: cleavage rate = Number of cleaved oocytes/total number of inseminated oocytes x 100.

Statistical analysis

The obtained data were statistically analysed by repeated measures analysis of variance (ANOVA) using SAS's General Linear Model procedure [26]. Differences among means were tested using the Range Multiple Test of Duncan [27]. All proportions were exposed to an arcsine transformation before the statistical analysis (chi-square analysis), and all differences were set at $P < 0.05$.

Results

Sperm motility and viability

As shown in Table 1, total and progressive motility of spermatozoa supplemented with 10 % and 15 % PRP were greater ($P < 0.05$) than their counterparts of 0 %, 5 %, and 20 % PRP whereas, their sperm viability was greater than those of 0 % and 20 % PRP and similar with that of 5 % PRP (Table 1).

The integrity of sperm cell membranes

The effect of different proportions of PRP supplementation on sperm cell membrane integrities is shown in Table 2. Frozen-stored spermatozoa in the presence of 15 % PRP had the greatest proportions of intact plasma (58.2 ± 0.9 %), acrosomal (76.3 ± 0.9 %), DNA (95.7 ± 0.7 %), and mitochondrial (56.3 ± 0.8 %) membranes integrities compared to the other proportions of PRP and the control group. All sperm cell membrane integrity was similar between the control group and the 20 % PRP group. Rather than mitochondrial membrane integrity, all sperm cell membrane integrities were similar between 5 % and 10 % PRP groups (Table 2).

Antioxidant activity and lipid peroxidation level

The incorporation of 15 % PRP into the freezing extender lead to the highest ($P < 0.05$) TAC, GPx, and SOD activities as well as the lowest MDA (0.4 ± 0.0 µM/mL) content compared to other groups. Moreover, the 5 % and 10 % PRP groups had higher SOD and GPx levels and lower MDA content than their counterparts in the control group. Surprisingly, TAC, SOD, GPx, and MDA levels of the 20 % PRP-treated group were similar to those of the control group (Table 3).

Cleavage rate (%)

As shown in Fig. 1, using 5 %, 10 %, and 15 % PRP-supplemented spermatozoa in the IVF experiment yielded a dose-dependent increase in oocyte cleavage rate with the greatest rate ($P < 0.05$, 59.2 ± 1.0 %) following IVF using 15 % PRP-supplemented frozen-thawed spermatozoa. Moreover, the obtained cleavage

rate was similar between the 20 % PRP and the control groups (Fig. 1).

TABLE 1.Motility and viability (mean ± SEM) of buffalo bull spermatozoa frozen-stored in the presence and absence of PRP

% PRP	Sperm motility (%)		Sperm viability (%)
	Total motility	Progressive motility	
0	63.0 ± 1.5^{bc}	59.0 ± 0.3^{d}	78.2 ± 1.6^{bc}
5	65.2 ± 0.8^{b}	61.1 ± 0.6^{c}	80.8 ± 1.1^{ab}
10	68.6 ± 0.8^{a}	63.1 ± 0.7^{b}	85.2 ± 1.8^{a}
15	69.9 ± 0.2^{a}	65. 0 ± 0.6^{a}	85.8 ± 2.0^{a}
20	60.0 ± 1.4^{c}	55.7 ± 0.9^{e}	75.3 ± 1.6^{c}

Within the same column (n = 36/treatment) means bearing one common superscript were similar (P ≥ 0.05).
PRP = Platelet-rich plasma.

TABLE 2. Membrane integrity (mean ± SEM) of buffalo bull spermatozoa frozen-stored in the presence and absence of PRP

% PRP	Sperm cell membranes integrities (%)			
	Plasma	Acrosome	DNA	Mitochondria
0	**48.2 ± 0.9^{c}**	**71.3 ± 0.9^{b}**	**90.2 ± 0.8^{bc}**	**48.5 ± 0.6^{d}**
5	**52.0 ± 0.9^{b}**	**73.2 ± 0.8^{b}**	**92.1 ± 0.5^{b}**	**51.2 ± 0.8^{c}**
10	**52.8 ± 0.7^{b}**	**72.0 ± 1.2^{b}**	**92.2 ± 0.7^{b}**	**53.0 ± 0.4^{b}**
15	**58.2 ± 0.9^{a}**	**76.3 ± 0.9^{a}**	**95.7 ± 0.7^{a}**	**56.3 ± 0.8^{a}**
20	**47.8 ± 0.9^{c}**	**70.5 ± 0.8^{b}**	**89.5 ± 0.9^{c}**	**49.3 ± 0.3^{d}**

Within the same column) n = 36/treatment) means bearing one common superscript were similar (P ≥ 0.05).
PRP = Platelet-rich plasma.

TABLE 3. Antioxidant activity and lipid peroxidation level of frozen-thawed buffalo semen supplemented and non-supplemented with PRP

% PRP	TAC (Mm)	GPx (nmol/min/mL)	SOD (U/mL)	MDA (µM/mL)
0	1.4 ± 0.1^{c}	15.8 ± 0.5^{c}	31.5 ± 0.7^{c}	1.6 ± 0.1^{a}
5	1.6 ± 0.1^{c}	22.0 ± 0.5^{b}	36.2 ± 1.6^{b}	0.9 ± 0.1^{b}
10	1.9 ± 0.1^{b}	24.0 ± 0.5^{b}	38.3 ± 1.9^{b}	0.7 ± 0.0^{b}
15	2.5 ± 0.1^{a}	27.0 ± 0.8^{a}	43.5 ± 1.8^{a}	0.4 ± 0.0^{c}
20	1.4 ± 0.1^{c}	17.2 ± 1.1^{c}	32.3 ± 0.8^{c}	1.5 ± 0.1^{a}

Within the same column) n = 45/treatment) means bearing one common superscript were similar (P ≥ 0.05).
PRP = Platelet-rich plasma, TAC = Total antioxidant capacity, GPx = Glutathione peroxidase, SOD = Superoxide dismutase, MDA = Malondialdehyde.

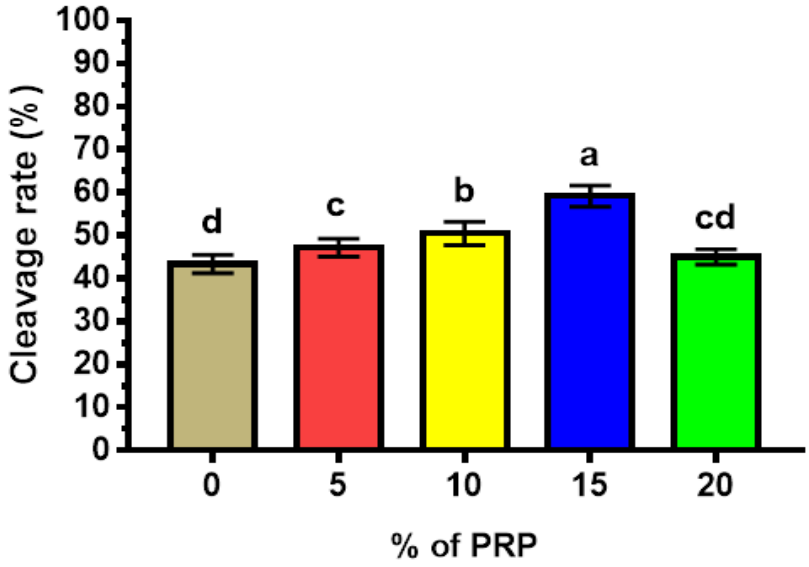

Fig. 1. Cleavage rate (mean ± SEM) following IVF of buffalo cow oocytes with buffalo bull spermatozoa frozen-stored in the presence and absence of PRP

The IVF capability of each group (n = 30) was evaluated by estimating the cleavage rate from the following equation: cleavage rate = No of cleaved oocytes/Total No of inseminated oocytes x 100.

Discussion

To enhance post-thaw sperm quality and fertility outcome, PRP was added to the semen extender to explore its possible impacts on buffalo bull spermatozoa. As far as we know, this investigation is the first to look at how autologous PRP addition to the semen extender affects the quality and *in vitro* fertility of frozen-thawed buffalo bull spermatozoa. We observed that adding 15 % PRP during cryopreservation of buffalo bull spermatozoa enhanced their post-thaw sperm motility and membrane integrity. This could be due to the buffering effect of PRP which avoids osmotic stress because its protein content mechanically preserves sperm membranes by decreasing the chance of crystallization or melting at various phases of the cryopreservation procedure [28]. Furthermore, PRP mitigates the freezing-thawing damage by preserving proteins of sperm membrane such as dermcidin acrosomal membrane-associated proteins, and calmodulin [6].

The presence of numerous bioactive components in PRP may be connected to its protective roles, as described by Saucedo et al. [29] who reported that FGF is a constituent in PRP, that enhances FGF receptor phosphorylation levels on sperm flagella and stimulates extracellular signal-regulated kinase and protein kinase B signalling pathways thus increase the percentages of both progressive and total sperm motility and some of sperm cell kinematics. In Harmony with our findings, Hernández-Corredor et al. [30] showed that incorporating autologous PRP into a ram semen extender enhances sperm morphometry and motility, which was attributed to the supportive action of FGF on sperm cell motility. The presence of numerous bioactive components in PRP may be connected to its protective roles, as reported by Saucedo et al. [29]. These authors group reported that FGF is a constituent in PRP that enhances FGF receptor phosphorylation on sperm flagella and stimulates extracellular signal-regulated kinase and protein kinase B signalling pathways. This results in an increased percentage of both progressive and total sperm motility, as well as improvements in some sperm cell kinematics.

Another plausible explanation for this increase in post-thaw sperm function and quality of buffalo bull semen obtained in the current study is the antioxidant action of IGF-1 [7]. IGF-1 is the main constituent of PRP and has been shown to enhance the integrities of the acrosomal membrane and sperm plasma membrane, as well as DNA integrity and mitochondrial membrane

potential. This was demonstrated when compared to a control group of human spermatozoa that did not receive PRP [4]. Additionally, IGF-1 boosts intracellular calcium ion levels by enhancing its transport, leading to improved progressive motility [31], and plays a key role in the energy metabolism of buffalo bull spermatozoa [32].

Herein and in our recent report [5], IGF-1 concentrations in PRP extracted from the blood of fertile buffalo bulls varied from 1350 to 2150 ng/mL, with an average of 1750 ng/mL. As a result, in the current study, 15 % PRP (≈250 ng/mL IGF-1) supplementation improved post-thaw sperm quality, increasing *in vitro* fertility of buffalo bull spermatozoa. This is consistent with the findings of Kumar et al. [33] who found that the post-thaw membrane integrity, viability, and motility of buffalo bull spermatozoa were enhanced by adding 250 ng/mL IGF-1 to buffalo semen before cryopreservation. Also, these findings emphasized our recent report that revealed supplementing of 15 % PRP to the semen extender was successful in increasing post-thaw antioxidant activity, quality, and *in vivo* fertility of buffalo bull spermatozoa [5].

Even though 20 % PRP should possess greater levels of IGF-1 than 15 % PRP it did not raise the quality of sperm and fertility, which could be due to greater quantities of PRP containing greater concentrations of serum, which leads to head-to-head agglutination of spermatozoa, in line with Dong et al. [34] This appears to be the most probable explanation, given that 20 % PRP had no positive effect on motility (progressive and total), viability, antioxidant activity, membrane integrity, and *in vitro* fertility of buffalo bull spermatozoa.

PRP has an anti-apoptotic and antioxidant function on mammalian cells; it may also assist in muscle regeneration by controlling antioxidant enzymes and decreasing radiation-induced anomalies in kidney histology [35]. Our recent research showed that adding 15 % PRP to semen extender dramatically raises SOD, GPx, and TAC levels while decreasing MDA levels in cryopreserved buffalo semen5]], denoting that PRP has high antioxidant activity in agreement with Bader et al. [9] who demonstrated that incorporating 2 % PRP was sufficient to repair the detrimental effects of H_2O_2-induced oxidative stress on human spermatozoa. Furthermore, Yan et al. [4] reported that human semen treated with PRP has low levels of reactive oxygen species (ROS) and a high proportion of intact mitochondrial membrane.

In this trial, 15 % PRP improved the *in vitro* fertility of buffalo bull spermatozoa, which may be related to PRP supplementation improving sperm membrane integrity including DNA integrity, because the fertility of buffalo bull spermatozoa was adversely affected by DNA denaturation [36]. In the present study, 15 % PRP supplementation yielded the greatest sperm DNA integrity (95.7 ± 0.7 %) in comparison with the control (90.2 ± 0.8 %) group. It is well known that freezing and thawing procedures lead to excessive ROS production which adversely affects all sperm cell membranes including DNA integrity [37]. This inevitable damage was not only eliminated through the addition of 15 % PRP to the freezing medium used for cryopreservation but also, improved all sperm membrane integrity including DNA integrity.

Conclusions

In conclusion, adding 15 % PRP to the freezing extender of buffalo semen is advised to improve their post-thaw sperm quality, antioxidant activity, and *in vitro* fertility. This study suggests a new methodology for enhancing the post-thaw quality and fertility of frozen-thawed buffalo bull spermatozoa.

Data availability

All relevant data are within the manuscript and any inquiry has to be sent to the corresponding author.

Conflicts of interest

There are no conflicts to declare.

Funding statement

This study was not funded by any governmental, private, or non-profit funding agencies.

Acknowledgements

The authors are grateful to all staff members at the International Livestock Management Training Center in Sakha, Egypt, for their unending support.

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تأثير إضافة البلازما الذاتية الغنية بالصفائح الدموية على جودة ما بعد الذوبان والخصوبة المعملية للحيوانات المنوية المجمده فى الجاموس

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يهدف هذا البحث على دراسة تأثير إضافة البلازما الذاتية الغنية بالصفائح الدموية الى المخفف المستخدم فى تجميد وحفظ نطفات السائل المنوى فى الجاموس وحمايتها من الأثار المترتبة على مراحل التجميد والحفظ وتأثير ذلك بالتبعية على الخصوبة لطلائق الجاموس. قمنا بتجميع 54 قذفه من تسعة طلائق جاموس، ثم تم تقسيم كل قذفه إلى 5 أجزاء متساوية ليتم تخفيفهم باستخدام مخفف Optixcell® مزود بخمسة تركيزات [0%، 5%، 10%، 15%، و 20%] من البلازما الذاتية الغنية بالصفائح الدموية متبوعة بـالتجميد بإستخدام البروتوكول المعتاد. تم تقييم حركة الحيوانات المنوية بعد الذوبان، وحيوية الحيوانات المنوية، ونسب سلامة كل من الغشاء البلازمي، والأكروسوم، والحمض النووي، وغشاء الميتوكوندريا. تم أيضًا قياس معدلات مضادات الأكسدة (SOD)، و (GPx)، وكذلك محتوى (MDA) و ناتج الاخصاب المعملي (IVF) لهذه الحيوانات المنوية المجمدة في وجود وعدم وجود البلازما الذاتية الغنية بالصفائح الدموية.

أدى إلى تحسين حيوية الحيوانات المنوية وحركتها التقدمية وسلامة غشاء البلازما. تركيزات البلازما الغنية بالصفائح الدموية بنسب 5% و10% و15% خفضت بشكل كبير كمية MDA وادت الي إرتفاع أنشطة انزيمي GPx وSOD. وتم زيادة معدل الخصوبة بشكل كبير بعد التلقيح المعملي بأستخدام الحيوانات المنوية المجمدة في وجود 15٪ من البلازما المنوية الغنية بالصفائح الدموية. في الختام، يوصى بإضافة 15% من البلازما الغنية بالصفائح الدموية إلى مخفف السائل المنوي المستخدم للتجميد في الجاموس لتحسين جودة ما بعد الذوبان، ونشاط مضادات الأكسدة ، وكذلك معدل الأخصاب المعملي.

الكلمات الدالة: الجاموس ، السائل المنوي المجمد ، البلازما الغنية بالصفائح الدموية.