

Platelet-rich plasma supplementation in semen extenders improves post-thaw sperm quality and fertility in buffalo (*Bubalus bubalis*): A systematic review and meta-analysis

Ragil Angga Prastiya ^{*1,2}, Trilas Sardjito ^{1,2}, Sri Pantja Madyawati ³, Muhammad Thohawi Elziyad Purnama ^{1,2,7}, Amung Logam Saputro ^{1,2}, Bodhi Agustono ^{1,2}, Nining Haryuni ⁴, Samira Musa Sasi ⁵, Nagia Musa Alghoul ⁶

¹ Department of Health and Life Sciences, Faculty of Health, Medicine, and Life Sciences (FIKKIA), Universitas Airlangga, Banyuwangi, Indonesia

² Research Group of Animal Biomedical and Conservation, Faculty of Health, Medicine, and Life Sciences, Universitas Airlangga, Banyuwangi, Indonesia

³ Department of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

⁴ Animal Science Department, Madani Indonesia University, Blitar, Indonesia

⁵ Department of Zoology, Faculty of Science, University of Tripoli, Tripoli, Libya

⁶ Libyan Center of Biotechnology Research, Tripoli, Libya

⁷ Department of Biology, Graduate School of Natural and Applied Sciences, Eskişehir Osmangazi Üniversitesi, Eskişehir, 26040, Türkiye

Article info

Received: 16 December 2025

Received in revised form: 07 March 2026

Accepted: 13 March 2026

Published online: 17 March 2026

Keywords

Buffalo

Platelet-rich plasma

Semen extender

Meta-analysis

Semen quality

Fertility

* Corresponding author:

Ragil Angga Prastiya

Email: ragilap@fkh.unair.ac.id

Reviewed by:

Alaa Kamil Abdulla

Department of Life Sciences, Faculty of Sciences-Sfax, University of Sfax, Tunisia

Consent to publish the name of second reviewer could not be obtained

Abstract

Cryopreservation of semen is an essential component of buffalo reproductive management, although the freeze-thaw process frequently induces sperm cryodamage and oxidative stress-related decline in fertility. Platelet-rich plasma (PRP) is a blood-derived concentrate rich in biologically active growth factors and endogenous antioxidants, which is a known enhancer of sperm cryosurvival. Therefore, it is supplemented in buffalo semen extenders. This systematic review and meta-analysis targeted to quantify the influence of PRP substitution in buffalo semen extenders on post-thaw sperm quality and fertility outcomes. A comprehensive literature search was conducted across five electronic databases to identify eligible controlled studies. PRP supplementation improves post-thaw sperm quality, including progressive motility, viability, plasma membrane integrity, and acrosomal integrity, while reducing malondialdehyde levels and increasing total antioxidant capacity and superoxide dismutase activity, indicating reduced oxidative damage during cryopreservation. Pooled fertility data revealed that the conception rates following insemination with PRP-enriched frozen semen were higher than those in the control group. There was moderate heterogeneity in the measured variables, which were largely attributable to the differences in PRP concentrations, preparation methods, and the types of extenders used rather than disagreement in the effect direction. The study results are consistent with earlier investigations, indicating that PRP may have different mechanisms of action of protecting sperm from freezing, which potentially translates into better reproductive performance after laboratory improvements. Although the current studies support the potential incorporation of PRP into buffalo semen cryopreservation protocols, further well-designed studies are required to define a standard method of PRP preparation, determine the optimum dose, and validate the effectiveness of PRP under field conditions.

This is an open access article under the CC Attribution license (<http://creativecommons.org/licenses/by/4.0/>)

1. Introduction

Artificial insemination (AI), together with semen cryopreservation, represents a fundamental pillar of breeding strategies in buffalo, especially in Asia, a continent where buffalo remains the backbone of the dairy sector and agricultural labor systems (Hufana-Duran et al. 2025). Genetically superior frozen semen allows the spread of desirable genes to a large number of animals over a wide geographic area, thereby shortening the intervals of genetic gains besides ensuring long-term sustainability through preservation and commercialization of the breeding stock (Ullah and Waheed 2025). However, fertility outcomes after AI with frozen-thawed buffalo semen mostly do not exceed the biological limits of the species, resulting in high financial losses at both farm and industry levels (El-Sherbiny et al. 2022). Cryodamage is a prime cause of physiological dysfunction in sperm cells, which is an

intricate process initiated by the freeze-thaw method (Khan et al. 2021). During cryopreservation, sperm are subjected to the formation of ice crystals, osmotic stress and cold shock, which can lead to plasma and acrosomal membrane ruptures, loss of mitochondrial membrane functions, and sperm DNA breakage (Ezzati et al. 2020). Furthermore, the freeze-thaw process drastically increases the generation of reactive oxygen species (ROS) that leads to oxidative stress, culminating in increased lipid peroxidation, depletion of the antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GSH), along with a decrease in sperm motility, viability, and fertilizing ability (Almadaly et al. 2023; El-Sherbiny et al. 2022). Experimental evidence suggests that the percentage of buffalo sperm cells losing their motility or viability during cryopreservation without any proper protective strategies might range from 30 to 60% (Almadaly et al. 2023).

To mitigate these effects, various antioxidants and other protective agents have been incorporated into semen extenders with variable degree of success (Bustani and Baiee 2021; Partyka and Nizański 2021). Due to its cytoprotective qualities, platelet-rich plasma (PRP) has been considered as a supplement in semen extenders. Being the platelet-enriched plasma fraction, PRP has a variety of growth factors and antioxidant molecules that can help sperm membranes to be stabilized, oxidative damage to be decreased, and mitochondrial function to be maintained, especially in the process of cryopreservation (Pavlovic et al. 2016; Abdulla et al. 2022; Yan et al. 2021; Tognoloni et al. 2023). From these processes, PRP is able to preserve sperm motility, membrane, and acrosome integrity after the freeze-thaw process - these parameters being recognized as critical for fertilization (Salama et al. 2024b). Research works on buffaloes have also shown that addition of PRP to semen extenders is capable of elevating several post-thaw sperm quality parameters which suggests the efficacy of PRP against cryo-injury and its ability to maintain sperm functional competence after cryopreservation. Experimental studies in buffalo indicate that PRP supplementation in semen extenders can improve post-thaw sperm quality and reproductive outcomes. For instance, 5% autologous PRP in a Tris-based extender enhanced progressive motility, membrane integrity, SOD and TAC activity, and reduced MDA levels, while also improving in vitro fertilization and embryo development rates (El-Sherbiny et al. 2022). Similarly, 15% PRP increased conception rates in sub-fertile buffalo bulls and improved antioxidant status (Almadaly et al. 2023). In another trial, 10% PRP combined with OptiXcell® improved motility, viability, TAC, GSH, and reduced MDA levels, accompanied by higher conception rates compared with the control group (Salama et al. 2024b).

Despite such promising potential, the differences in methods of application, PRP source such as autologous versus heterologous, methods of preparation, PRP concentrations (2–25%), types of extenders used, and freezing curves hinders the development of an optimal PRP dosage and application protocol (Alcay et al. 2022; Salama et al. 2024a; Yan et al. 2021). Buffalo semen cryopreservation is one of the few areas where current research about PRP supplementation is still varied due to factors like experimental design, PRP preparation method, extender composition, and outcome measurement differences

that don't allow direct comparison or give a solid basis for artificial insemination recommendations. Even though there are positive results, no quantitative summary has been done to assess the general effectiveness of PRP in this area. The paper offers a first systematic review and meta-analysis to quantitatively analyze the effects of PRP supplementation in buffalo semen extenders on post-thaw sperm quality and fertility parameters while identifying the reasons for differences.

2. Methods

2.1. Protocol and registration

The entire protocol has been registered and publicly available on the Open Science Framework (OSF) (<https://doi.org/10.17605/OSF.IO/2CG45>). The choice of OSF was driven by the fact that it offers permanent archiving, version-control, and a complete audit trail of any protocol amendments. The uploaded protocol provides a detailed account of the literature search strategy, study selection criteria, predefined primary and secondary outcomes, statistical procedures for quantitative synthesis, and methods for dealing with inconsistent or missing data. Specifying these aspects beforehand essentially removes the risk of post-hoc bias and retains methodological integrity during the entire review. Reporting of this review is based on the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) standards.

2.2. Inclusion criteria (PICOS framework)

Study eligibility was established by using the PICOS framework to guarantee biological and methodological appropriateness. Only research articles which strictly adhered to the predetermined population, intervention, comparator, outcome, and study design criteria were allowed to be included in the review. This method preserves some standard fluctuation in cryobiology and reproductive biotechnology experiments while ensuring enough discipline for the studies to be synthesizable both qualitatively and quantitatively. The details of the PICOS criteria applied in this review are given in Table 1.

2.3. Search strategy

An extensive literature search across was conducted across five major

Table 1. PICOS criteria for study inclusion		
PICOS	Inclusion criteria	Exclusion criteria
Population	Buffalo semen (<i>Bubalus bubalis</i>), all breeds (Murrah, Nili-Ravi, Surti, local). Buffalo-specific data must be available separately.	Studies without buffalo data; non-extractable data; non-Bovidae species.
Intervention	Supplementation of PRP (autologous or heterologous) into semen extenders prior to cryopreservation; variations in PRP concentration and preparation methods accepted.	Non-PRP treatments; PRP added only after thawing; PRP without a control comparator.
Comparison	Extender without PRP (control), or standard alternative extenders.	Absence of a control group; pre–post designs without a proper comparator.
Outcomes – <i>In Vitro</i>	Progressive motility, viability, plasma membrane integrity, acrosome integrity.	Irrelevant outcomes; non-quantitative data; absence of SD/SE or replication number.
Outcomes – <i>In Vivo</i>	Conception or pregnancy rate via artificial insemination (AI).	Fertility studies without AI; pregnancy outcomes not reported numerically.
Outcomes – Antioxidant	MDA, TAC, SOD.	Biomarkers unrelated to oxidative stress or not convertible to quantitative form.
Study Design	In vitro or in vivo experimental studies with control groups; randomized or non-randomized controlled trials.	Reviews, editorials, opinion papers; case reports; conference abstracts without full data.
Abbreviations: PICOS: Population, Intervention, Comparison, Outcomes, and Study design; PRP: Platelet-rich plasma; SD: standard deviation; SE: Standard error; AI: Artificial insemination; MDA: Malondialdehyde; TAC: Total antioxidant capacity; SOD: Superoxide dismutase		

electronic scientific databases: Scopus, PubMed, Web of Science, Google Scholar, and ScienceDirect. Only articles written in English were included, and eligible non-English studies were translated. The search covered all the available years in each database until the final search date (23 November 2025).

The search strategy combined controlled vocabularies (such as MeSH terms in PubMed) and free-text terms related to platelet-rich plasma (PRP), semen preservation, and buffalo species. Below is an instance of the search string used in PubMed database: (("platelet rich plasma" OR PRP OR "platelet-rich plasma") AND (semen OR spermatozoa OR sperm OR cryopreserve OR cryopreservation OR "frozen semen" OR extender) AND (buffalo OR bubalus OR "water buffalo" OR "*Bubalus bubalis*")). The search strategy was adapted according to the syntax and indexing systems of each database. Additionally, the reference lists of all included articles and relevant review papers were manually screened to identify further eligible studies.

2.4. Study selection and data extraction

Study selection was made through two main phases: (1) title and abstract screening, and (2) full-text review. The screening procedure was carried out by two independent reviewers, and differences, if any, were resolved through discussion or a third reviewer was consulted, wherever necessary. The extracted variables included the author and year of publication, number of semen samples or ejaculates analyzed, type of platelet-rich plasma (PRP) preparation, PRP concentration used in the semen extender, type of semen diluent/extender, freezing and cryopreservation protocol, and the evaluated post-thaw sperm quality parameters. When multiple PRP concentrations were tested within a single study, each comparison with the control group was recorded as an independent dataset for the meta-analysis.

The systematic search executed on 23 November 2025 in five leading databases (Scopus, PubMed, Web of Science, ScienceDirect, and Google Scholar) identified 21 records and 12 more records among the reference lists of the studies through manual screening (total 33). After the removal of 12 duplicates, 21 unique records remained for title and abstract screening. At this stage, 17 studies were excluded because they were not directly related to PRP application in buffalo semen cryopreservation. Subsequently, 16 full-text articles were assessed for eligibility according to the predefined PICOS criteria. Of these, 12 studies were excluded due to the absence of PRP supplementation in

semen extenders (intervention criteria), lack of extractable quantitative data (outcome criteria), use of non-buffalo species (population criteria), or absence of a control group (comparator criteria). Finally, four studies met the inclusion criteria and were included in the qualitative synthesis. These studies contained six independent experimental comparisons evaluating different PRP concentrations or extender formulations, and these comparisons were treated as separate datasets in the quantitative meta-analysis (Table 2). The study selection process is illustrated in the PRISMA Flow Diagram (Fig. 1).

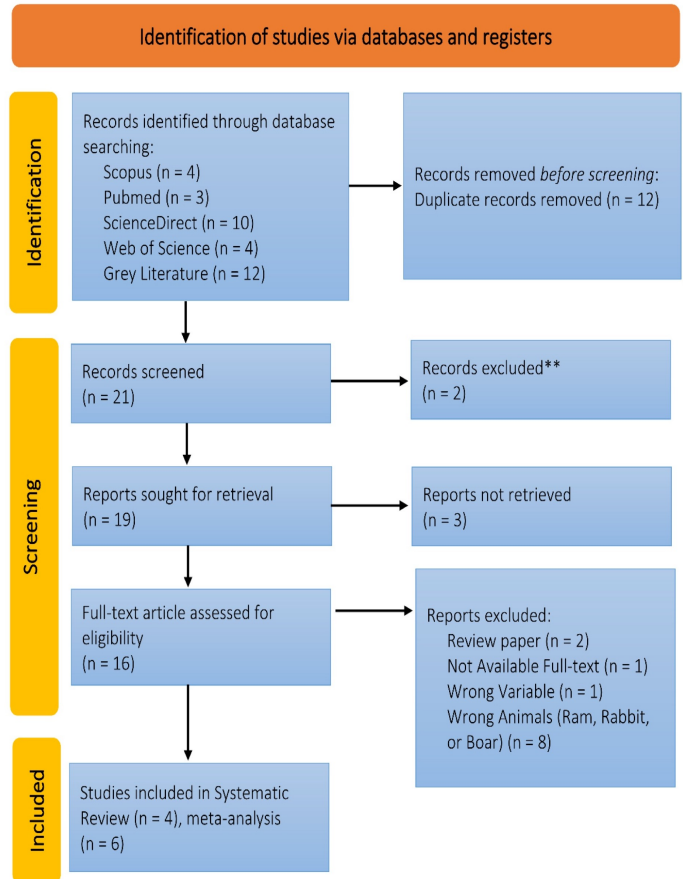


Fig. 1. PRISMA flow diagram summarizing the selection process from initial identification to final study inclusion

Table 2. Characteristics of studies included in the systematic review and meta-analysis							
Author	Country	Bull (no.)	Ejaculates (no.)	Extender	PRP source & preparation	PRP concentration	Outcomes evaluated
Almadaly et al. 2025	Egypt	9	36	OptiXcell®	Autologous PRP; double centrifugation (300g 5 min + 700g 17 min)	0–20%	Motility (CASA), viability, membrane integrity, acrosome integrity, DNA integrity, MMP, TAC, SOD, GPx, MDA, IVF cleavage rate
Salama et al. 2024	Egypt	10	48	OptiXcell® and Tris yolk	Autologous PRP; double centrifugation (300g + 700g) + Ca-gluconate activation	0–15%	Motility, viability, abnormalities, membrane & acrosome integrity, MMP (JC-10), GSH, TAC, MDA, in vivo conception rate
Almadaly et al. 2023	Egypt	11	60	Tris yolk	Autologous PRP, two-step centrifugation	0–25%	Post-thaw motility, viability, HOST, acrosome status, oxidative markers
El-Sherbiny et al. 2022	Egypt	8	40	TCFY	Autologous PRP	0–10%	Motility, membrane integrity, oxidative stress parameters
Abbreviations: PRP: Platelet-rich plasma; CASA: Computer-assisted sperm analysis; MMP: Mitochondrial membrane potential; TAC: Total antioxidant capacity; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde; IVF: In vitro fertilization; MMP (JC-10): Mitochondrial membrane potential fluorescent probe; GSH: Glutathione; HOST: Hypo-osmotic swelling test; TCFY: Tris–citric acid–fructose–egg yolk extender							

2.5. Quality assessment

The methodological quality and risk of bias of the included studies were assessed using a structured domain-based approach inspired by the Cochrane risk-of-bias framework. Each domain was evaluated and categorized as “low risk,” “unclear risk,” or “high risk” of bias based on predefined signaling questions. The assessment was independently performed by two reviewers for all included studies. However, because most included studies were laboratory-based semen cryopreservation experiments rather than randomized controlled trials, certain domains related to randomization and allocation concealment were not always applicable. Consequently, the results of the bias assessment should be interpreted with caution. Although tools specifically developed for animal experimental studies, such as the SYRCLE risk-of-bias framework, may provide a more tailored approach for animal intervention research, the present review employed a generalized domain-based framework to systematically identify potential sources of bias across the included studies.

2.6. Statistical analysis

All statistical analyses were performed using RevMan software, version 5.4 (Cochrane Collaboration, London, UK) to carry out all the statistics. For continuous outcomes such as sperm motility, viability, membrane integrity, acrosome integrity, and antioxidant biomarkers, we reported mean differences (MD) with 95% confidence intervals when the included studies used comparable measurement units. In several outcomes, mean differences were calculated as control minus experimental values; therefore, negative MD values indicate higher values in the PRP-treated group and were interpreted as improvements in sperm quality parameters. Risk ratios (RR) with 95% confidence intervals were reported for dichotomous outcomes such as conception rate or pregnancy rate. For antioxidant biomarkers, minor methodological variations were observed across studies. Superoxide dismutase (SOD) activity was reported using comparable measurement units across the included studies; therefore, pooling using mean difference was considered appropriate. In contrast, total antioxidant capacity (TAC) was measured using different assay systems and reporting units among studies. Although standardized mean difference (SMD) is typically recommended when measurement scales differ, mean difference was retained to preserve biological interpretability of the effect size. Consequently, TAC results were interpreted cautiously due to potential methodological variability across assays.

Since the included studies were different in terms of PRP preparation methods, extender formulations, cryopreservation protocols, and experimental conditions, to address the possibility of such heterogeneity among studies, a random-effects model was used in all meta-analyses. I^2 , a statistic for measuring the proportion of variation in study estimates that is due to heterogeneity rather than sampling error, was used to quantify heterogeneity between studies. Roughly, I^2 values of 25%, 50%, and 75% correspond to low, moderate, and high levels of heterogeneity, respectively. Because the number of available studies for several outcomes was limited, formal meta-regression or extensive sensitivity analyses were not always feasible; therefore, heterogeneity was interpreted qualitatively based on methodological differences among studies. When there were enough comparisons, a visual examination of the funnel plot and Egger's regression test were used to assess publication bias both statistically and graphically.

3. Results

3.1. Study characteristics

Four publications satisfied the criteria for inclusion and were therefore incorporated into the systematic review's qualitative synthesis. Those four investigations produced six experimental comparisons evaluating different PRP concentrations or extender formulations, which were utilized as separate datasets in the quantitative meta-analysis. All studies were carried out in Egypt between 2022 and 2025, and they all focused on the use of autologous platelet-rich plasma (PRP) in semen extenders for buffaloes prior to cryopreservation. In spite of the fact that there were methodological differences between studies, especially in terms of the concentrations of PRP, formulations of the extenders, and the range of functional and biochemical parameters assessed, the overall experimental design was broadly similar. All the studies collected semen from breeding bulls, prepared PRP by double centrifugation, applied PRP at different concentrations, and assessed sperm quality after freezing. Studies varied in their reporting of sample size. Two studies (Almadaly et al. 2025; Salama et al. 2024a) clearly mentioned the number of bulls and ejaculates (36 and 48 ejaculates, respectively), while the other two studies (Almadaly et al. 2023; El-Sherbiny et al. 2022) used pooled ejaculates (60 and 40 ejaculates, respectively) without clearly mentioning the number of ejaculates per replication. The extenders used were different and included OptiXcell®, Tris-egg yolk, and Tris-citrate-fructose-yolk (TCFY), representing both commercial and conventional formulations. Autologous PRP was prepared by a two-step centrifugation method in all cases, while one study also activated PRP with calcium gluconate before adding it to the extender (Salama et al. 2024b).

Different concentrations of PRP were investigated, ranging from 0–10% (El-Sherbiny et al. 2022) to 0–25% (Almadaly et al. 2023), although most studies evaluated moderate levels of supplementation (5%, 10%, and 15%). After thawing, the main functional parameters of sperm typically assessed were total and progressive motility, viability, membrane and acrosome integrity, and hypo-osmotic swelling test. Some of the researchers also looked into the oxidative stress by analyzing the level of malondialdehyde (MDA), antioxidant status was also determined by measuring total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione (GSH), along with mitochondrial membrane potential. Two studies additionally reported fertility outcomes such as *in vitro* fertilization parameters or conception rates after artificial insemination, thus providing an additional and important information on reproductive performance.

3.2. Quality assessment

The methodological quality of the included studies was evaluated using a structured domain-based risk-of-bias assessment approach. Overall, the methodological quality was considered moderate. Most studies clearly described semen collection procedures, PRP preparation protocols, extender composition, and laboratory assessment methods, resulting in low risk of bias for outcome measurement and completeness of reported data. However, several methodological limitations were identified. In particular, randomization procedures for allocating ejaculates to treatment groups were rarely described in detail, resulting in an “unclear risk” classification for the sequence generation domain in most studies. Similarly, blinding of laboratory assessors was generally not reported, which also resulted in an “unclear risk” rating

for detection bias.

One study presented a potential risk of selective outcome reporting, as not all evaluated parameters were consistently reported across all PRP concentrations, and subgroup fertility results lacked complete methodological description. Apart from this instance, no study was classified as having a high risk of bias in the core methodological domains. Because many of the included studies were laboratory-based semen cryopreservation experiments rather than randomized controlled trials, certain domains related to randomization and allocation concealment were frequently rated as unclear due to insufficient reporting. Overall, most studies were categorized as presenting low-to-unclear risk of bias, highlighting acceptable methodological quality but also indicating the need for improved reporting of randomization procedures, assessor blinding, and experimental unit definition in future studies (Fig. 2).

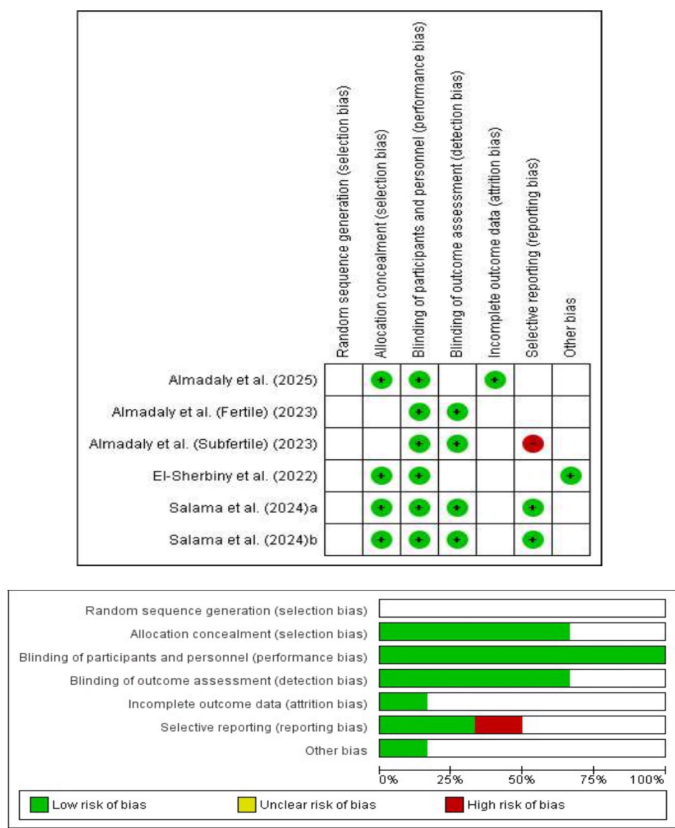


Fig. 2. Summary and graphical distribution of risk-of-bias judgments across the included studies using the Cochrane ROB-2 tool. Most studies demonstrated low risk across core domains, with several unclear ratings due to incomplete reporting of allocation procedures and blinding

3.3. Post-thaw sperm quality parameters

3.3.1. Progressive motility

Four studies providing six independent comparisons evaluated the effect of PRP supplementation on post-thaw progressive motility of buffalo semen. The pooled meta-analysis demonstrated a significant overall increase in progressive motility in PRP-treated groups compared with controls (MD = 4.66; 95% CI: 1.66–7.67). A particularly large effect was observed in one comparison evaluating 5% PRP supplementation, which showed a substantial increase in progressive motility (MD = 16.96; 95% CI: 6.57–27.35; P = 0.001) (El-Sherbiny et al.

2022). Three comparisons from two studies evaluated 10% PRP supplementation, showing a moderate but statistically non-significant improvement in progressive motility (MD = 3.62; 95% CI: –1.72–8.97) with moderate heterogeneity (I² = 70%), which may reflect differences in extender composition and PRP preparation protocols (Almadaly et al. 2023; Salama et al. 2024a). Two comparisons evaluating 15% PRP also indicated a positive trend toward increased progressive motility (MD = 5.41; 95% CI: –4.61–15.42) with moderate heterogeneity (I² = 39%). Although the effect was not statistically significant at this concentration, both comparisons showed numerically higher motility values in PRP-treated samples. Overall, PRP supplementation was associated with improved post-thaw progressive motility, although the magnitude of effect varied across studies and PRP concentrations. The corresponding forest plot is presented in Fig. 3.

3.3.2. Viability

Sperm functional parameters most regularly examined after thawing are sperm total and progressive motility, viability, membrane integrity, acrosome integrity, and sperm that respond to hypo-osmotic swelling. Some research works took the measure of oxidative stress through the detection of malondialdehyde (MDA), along with the evaluation of antioxidant status by determining total antioxidant capacity, SOD, GPx, and GSH, in addition to mitochondrial membrane potential. Combined analysis of four studies has revealed that the post-thaw sperm viability was significantly higher in the groups treated with PRP than in the control groups (MD = 5.41; 95% CI: 2.43–8.39; P = 0.007) (Fig. 4). A test for subgroup differences showed significant variation among PRP concentrations (χ² = 10.61, P = 0.005). The overall heterogeneity across comparisons was moderate (I² = 42%), implying that experimental conditions such as extender type and semen quality may change treatment effects.

3.3.3. Plasma membrane integrity

The meta-analysis uncovered that treatment with PRP leads to a considerable improvement in plasma membrane integrity in spermatozoa in contrast to controls (MD = –9.88; 95% CI: –11.25 to –8.52; P < 0.00001) (Fig. 5). For several outcomes, mean differences were calculated as control minus experimental values. Therefore, negative MD values indicate higher values in the PRP-treated group and were interpreted as an improvement in sperm quality parameters. Each PRP concentration subgroup exhibited a protective effect on the membrane with minimal variation between studies (I² = 0%). Among the groups, the 15% PRP subgroup provided the most stable results in terms of improvement (MD = –10.00; 95% CI: –12.49 to –7.51). By these data, it can be argued that PRP treatment helps maintain sperm plasma membrane integrity during the freezing process.

3.3.4. Acrosome integrity

Supplementation with PRP led to enhanced acrosome integrity as compared to the control group (MD = –6.17%; 95% CI: –9.41 to –2.93; P = 0.004) (Fig. 6). Subgroup with 5% PRP exhibited the most significant effect size (MD = –13.00; 95% CI: –17.80 to –8.20). Cases with 10% PRP revealed mixed outcomes (MD = –5.72; 95% CI: –12.48 to 1.05) along with fairly high heterogeneity (I² = 75%). 15% PRP subgroup changes were positive and very homogeneous (I² = 0%; MD = –5.00; 95% CI: –5.00 to –5.00). In agreement with different extenders used, quality of semen, and other different ways of PRP preparation, diversity in results was confirmed by 73% I² estimate.

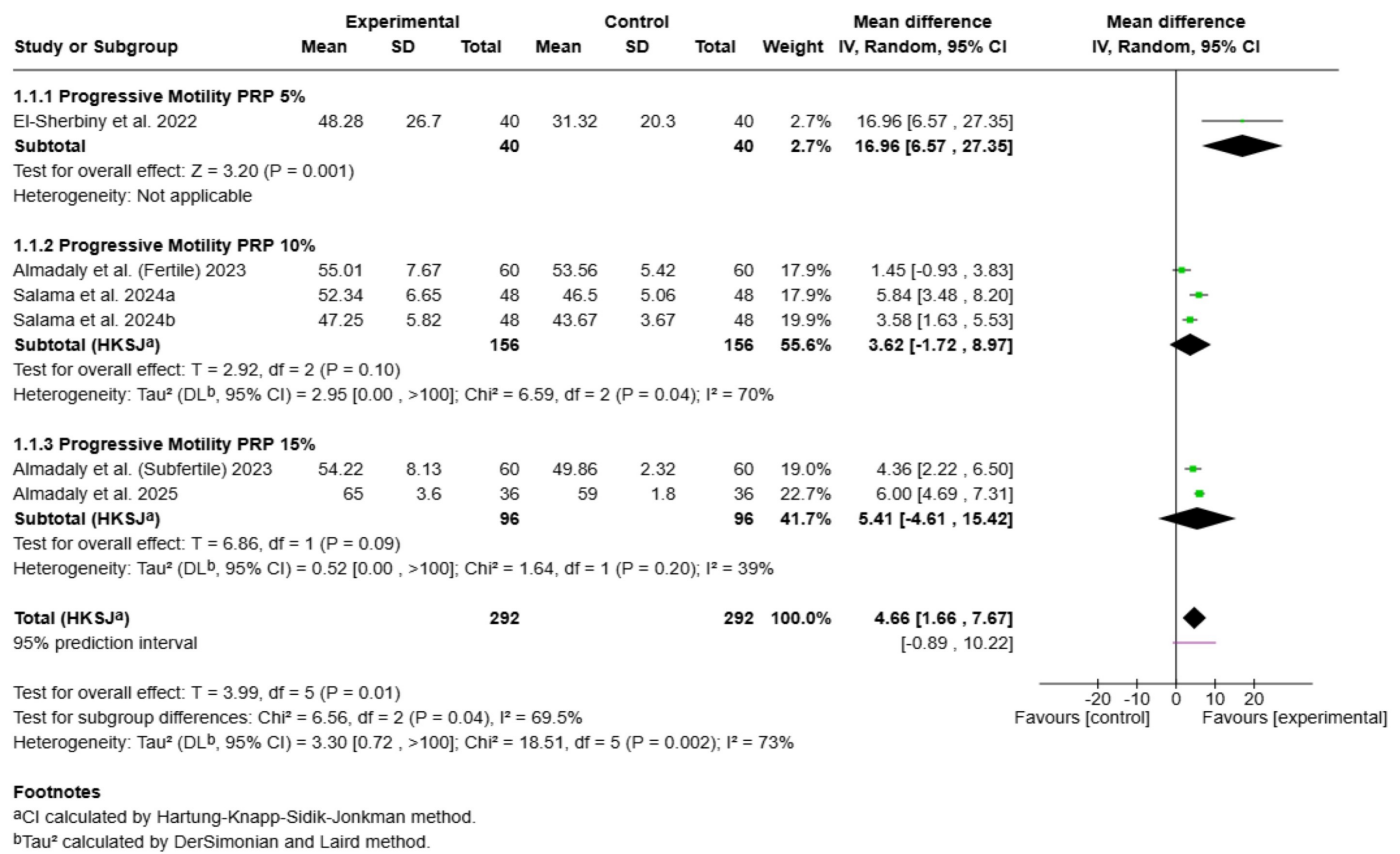


Fig. 3. Forest plot of meta-analysis on the effect of platelet-rich plasma (PRP) supplementation in semen extender on progressive motility (%) of post-thaw buffalo spermatozoa with 95% CI. Mean difference (MD), standard deviation (SD), and total sample size (Total) of PRP-treated and control groups are presented

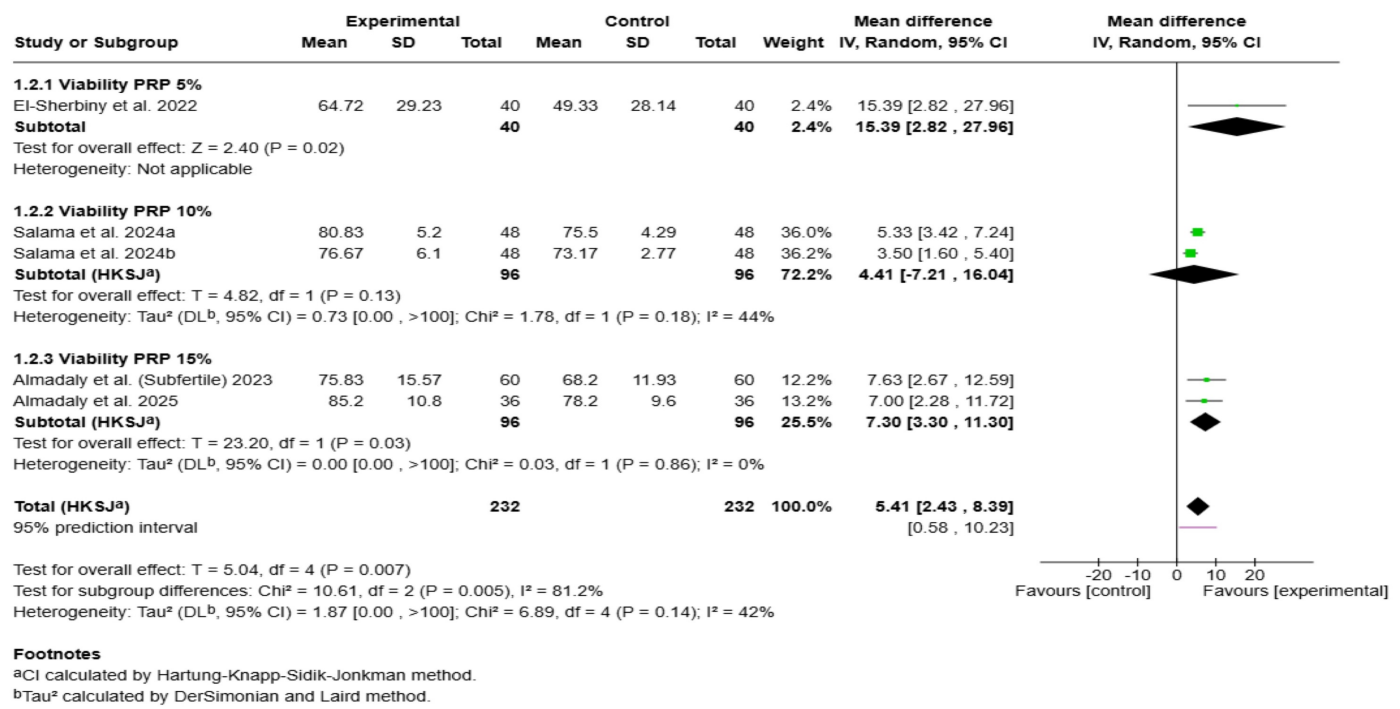


Fig. 4. Forest plot of meta-analysis on the effect of platelet-rich plasma (PRP) supplementation in semen extender on viability (%) of post-thaw buffalo spermatozoa with 95% CI. Mean difference (MD), standard deviation (SD), and total sample size (Total) of PRP-treated and control groups are presented

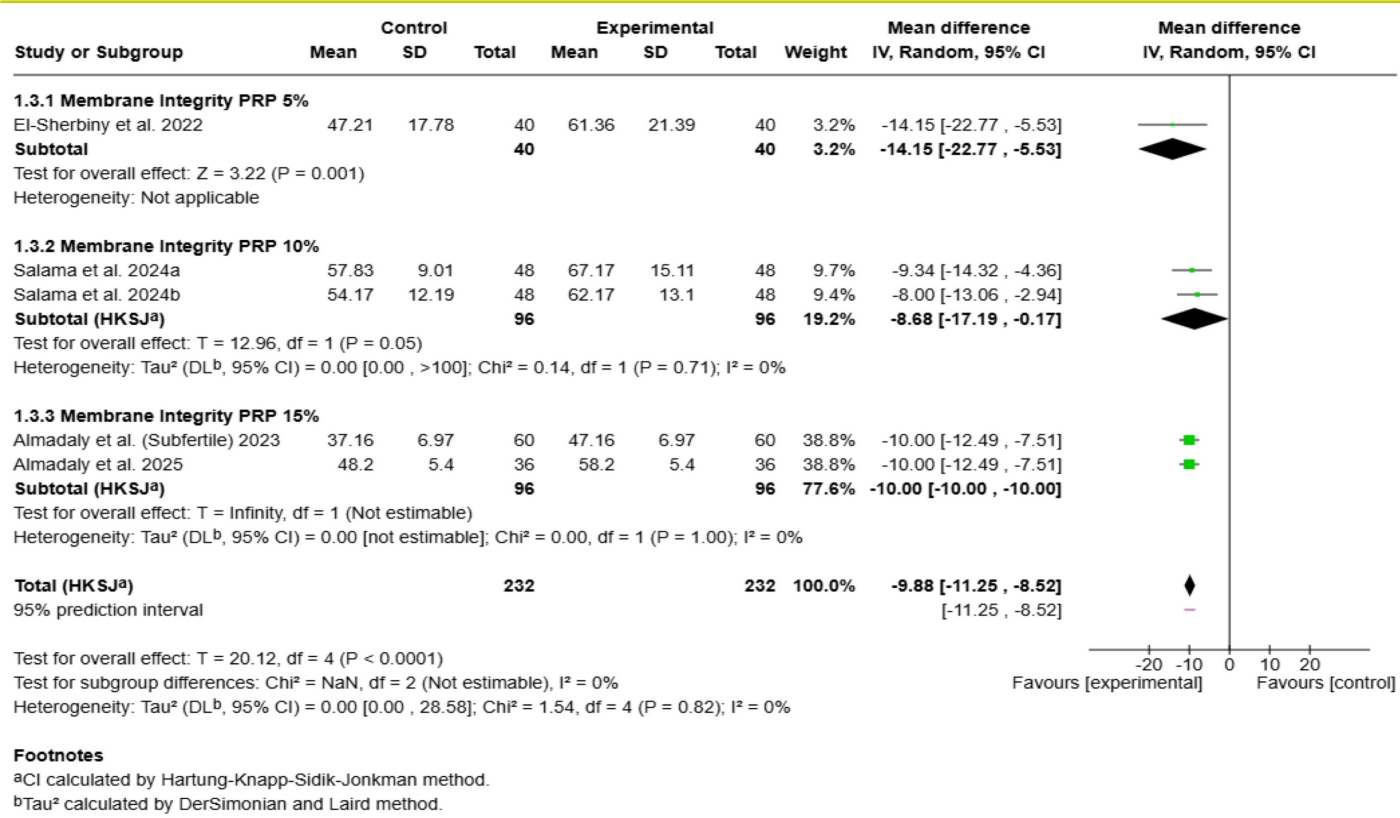


Fig. 5. Forest plot of meta-analysis on the effect of platelet-rich plasma (PRP) supplementation in semen extender on plasma membrane integrity (%) of post-thaw buffalo spermatozoa with 95% CI. Mean difference (MD), standard deviation (SD), and total sample size (Total) of PRP-treated and control groups are presented

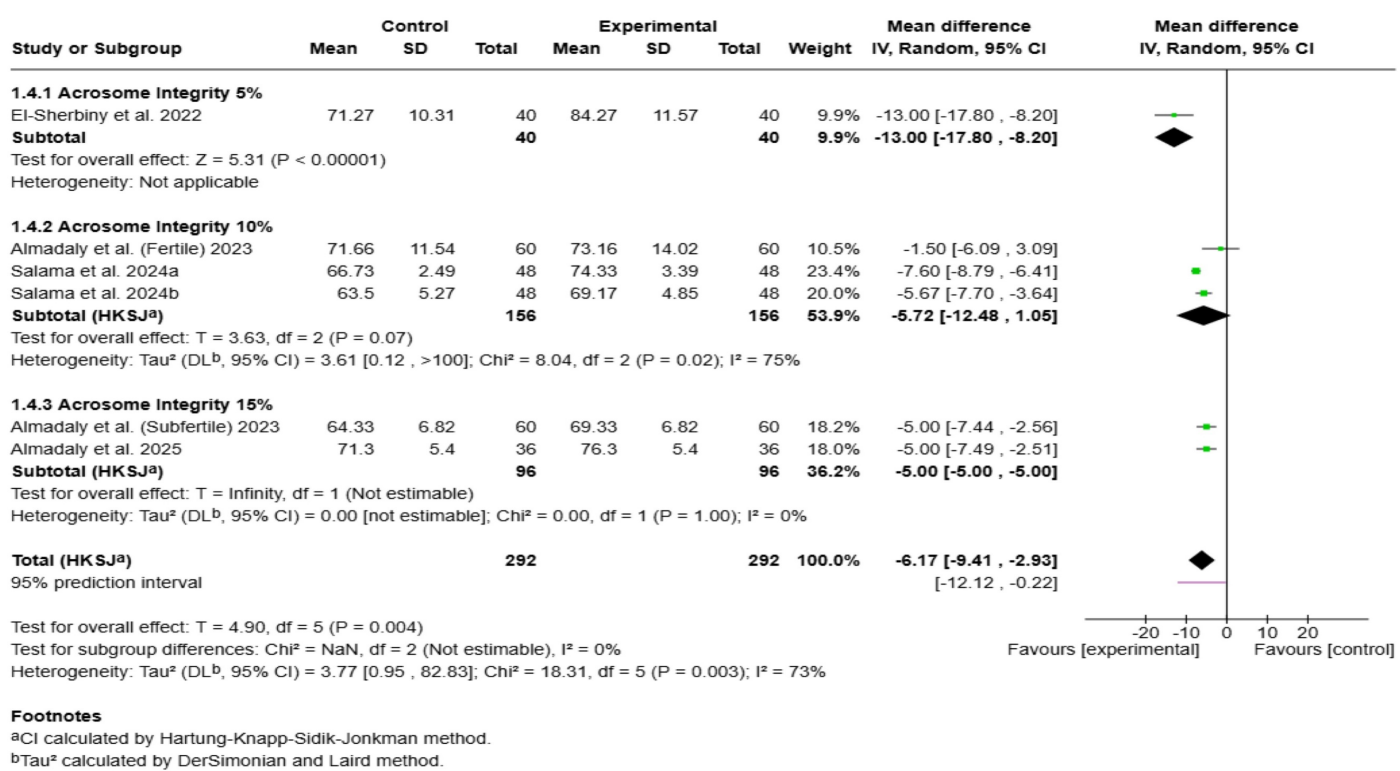


Fig. 6. Forest plot of meta-analysis on the effect of platelet-rich plasma (PRP) supplementation in semen extender on acrosome integrity (%) of post-thaw buffalo spermatozoa with 95% CI. Mean difference (MD), standard deviation (SD), and total sample size (Total) of PRP-treated and control groups are presented

3.4. Oxidative stress and antioxidant markers

3.4.1. Malondialdehyde (MDA)

Three comparisons checked the influence of PRP addition on frozen-thawed malondialdehyde (MDA) levels (Fig. 7). PRP-supplemented samples exhibited lower MDA levels on average than the untreated ones, thus reflecting less lipid peroxidation. Nevertheless, the combined result was not statistically significant (MD=1.19; 95% CI: -0.75 to 3.12; P = 0.08). Some differences (moderate heterogeneity) were revealed (I² = 48%) and could be related to variations in PRP activation methods, extender compositions, and initial oxidative status.

3.4.2. Total antioxidant capacity

Meta-analysis of four separate studies showed that the total antioxidant capacity was significantly increased in the samples with PRP compared to the controls (MD = -0.88; 95% CI: -1.61 to -0.14; P = 0.03) (Fig. 8). The 5% PRP group experienced a small but statistically significant effect (MD = -0.19; 95% CI: -0.27 to -0.11), whilst the 15% PRP group showed a very significant increase in the total antioxidant capacity (MD = -1.12; 95% CI: -1.16 to -1.07). The studies exhibited very high heterogeneity (I² = 99%), which is probably due to differences in the concentration of PRP, the composition of the extender, and the initial antioxidant status of the samples.

3.4.2. Superoxide dismutase (SOD)

According to a meta-analysis, the level of antioxidant enzyme superoxide dismutase (SOD) was significantly higher in the platelet-rich plasma (PRP) treated group than in the control group (MD = -12.31; 95% CI: -16.59 to -8.04; P = 0.003) (Fig. 9). Both 5% PRP and 15% PRP subgroups indicated highly positive effects, although there was

moderate heterogeneity among the studies (I² = 46%). Further analysis by subgroups showed that the difference in antioxidant response may be dose-dependent as the difference between PRP concentrations was statistically significant ($\chi^2 = 5.54$, P = 0.02).

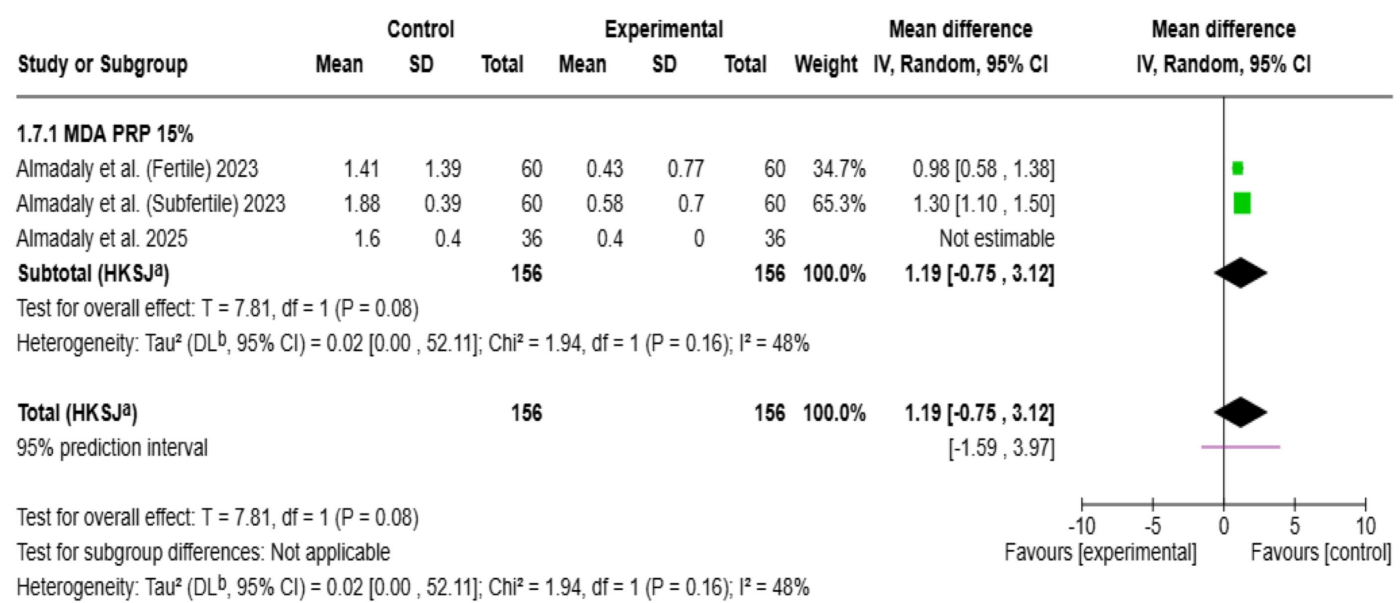
3.5. Fertility outcomes

3.5.1. Conception/Pregnancy rate

Only two studies reported in vivo fertility outcomes, providing six experimental comparisons for the quantitative synthesis. The pooled analysis revealed that PRP-treated groups experienced a higher conception rate than controls (RR = 1.51; 95% CI: 1.29–1.76; P = 0.001) (Fig. 10). Analysis by subgroup demonstrated that 5% PRP had a positive effect, although this was not statistically significant (RR = 1.57; 95% CI: 0.95–2.61). The 10% PRP subgroup yielded a point estimate that was not statistically significant and had extremely wide confidence intervals (RR = 1.83; 95% CI: 0.18–18.25), which suggests low precision due to the small number of observations. On the other hand, the 15% PRP subgroup displayed a statistically significant rise in conception rate (RR = 1.45; 95% CI: 1.01–2.07). There wasn't any major heterogeneity detected among the included comparisons (I² = 0%). However, since fertility outcomes were only available from two studies, the results should be taken very cautiously.

3.6. Publication bias

In order to detect potential publication bias in the analyzed outcomes, a funnel plot examination was performed. Nevertheless, a detailed assessment of funnel plot asymmetry, such as Egger's regression test, was not carried out since, for each outcome, the number of studies included was less than ten, which renders the reliability of such tests doubtful. At first glance, the funnel plots did not display obvious



Footnotes

^aCI calculated by Hartung-Knapp-Sidik-Jonkman method.

^bTau² calculated by DerSimonian and Laird method.

Fig. 7. Forest plot of meta-analysis on the effect of platelet-rich plasma (PRP) supplementation in semen extender on MDA levels (μM/mL) of post-thaw buffalo spermatozoa with 95% CI. Mean difference (MD), standard deviation (SD), and total sample size (Total) of PRP-treated and control groups are presented

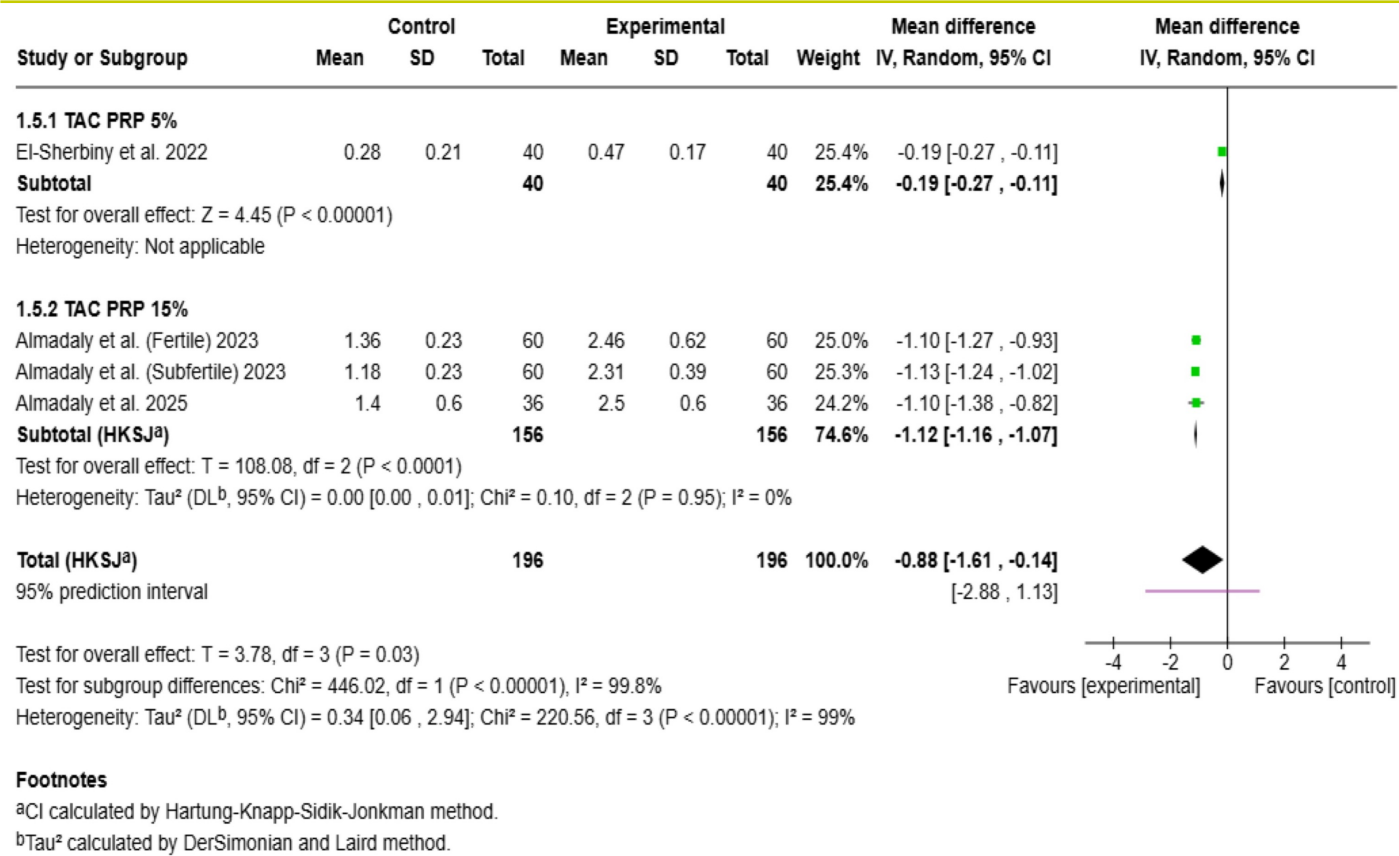


Fig. 8. Forest plot of meta-analysis on the effect of platelet-rich plasma (PRP) supplementation in semen extender on Total Antioxidant Capacity (Mm) of post-thaw buffalo spermatozoa with 95% CI. Mean difference (MD), standard deviation (SD), and total sample size (Total) of PRP-treated and control groups are presented

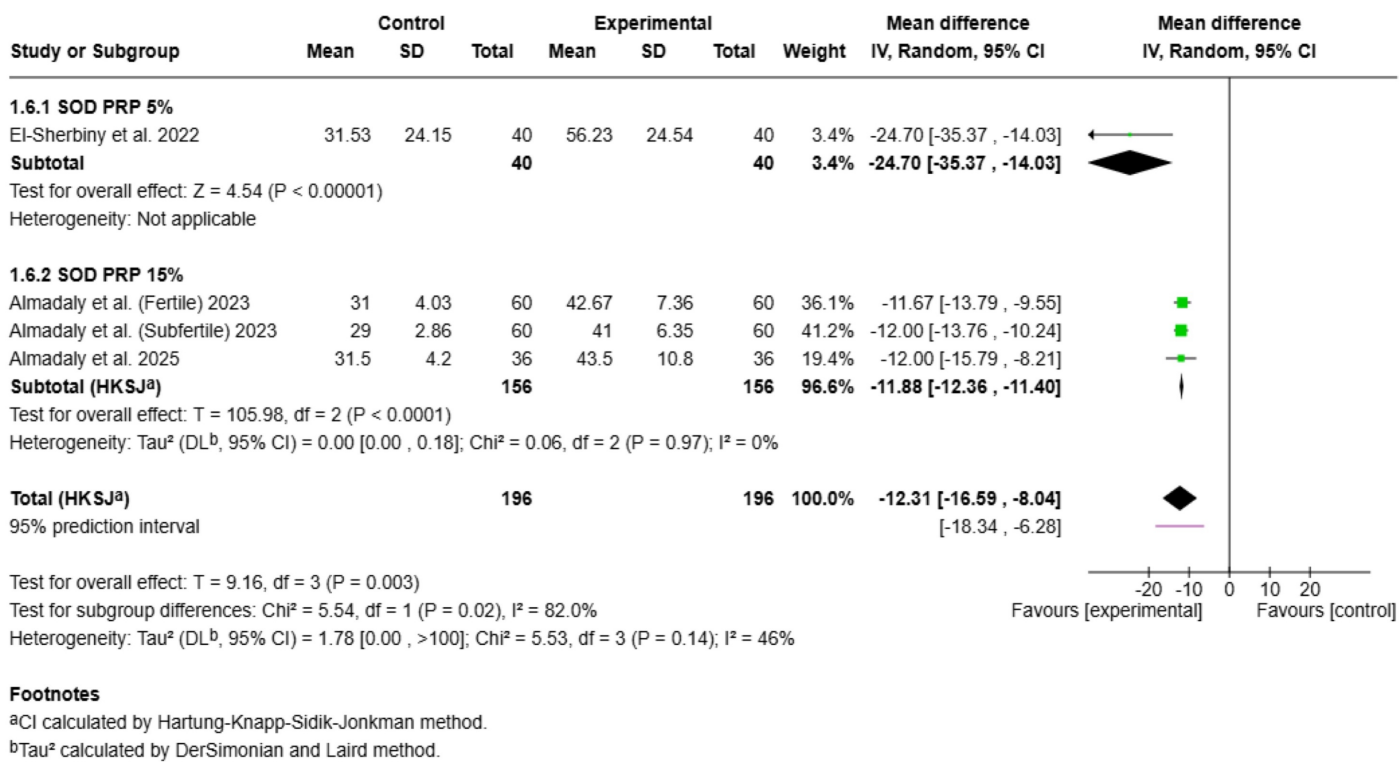


Fig. 9. Forest plot of meta-analysis on the effect of platelet-rich plasma (PRP) supplementation in semen extender on SOD levels (U/mL) of post-thaw buffalo spermatozoa with 95% CI. Mean difference (MD), standard deviation (SD), and total sample size (Total) of PRP-treated and control groups are presented

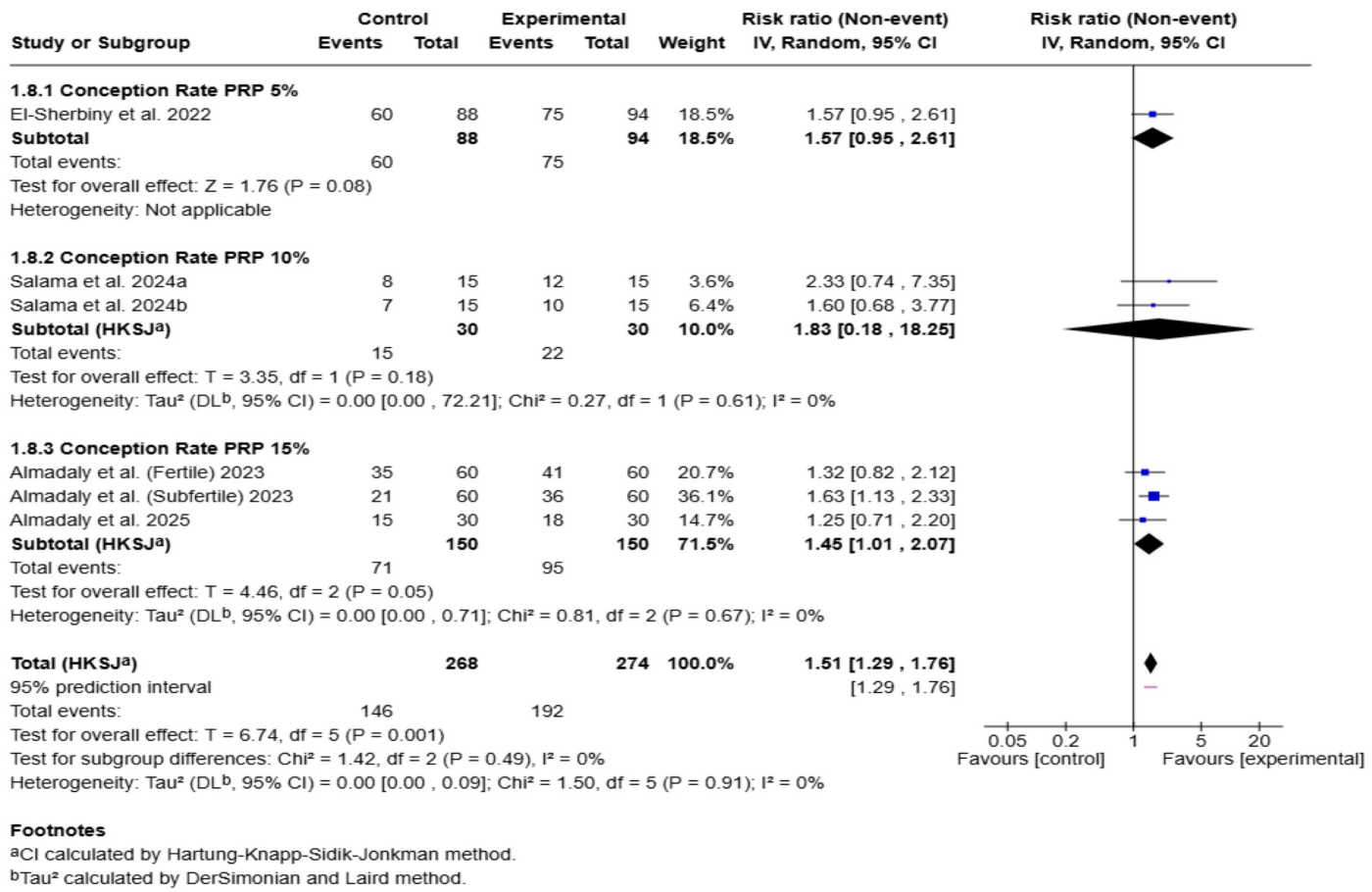


Fig. 10. Forest plot of meta-analysis on the effect of platelet-rich plasma (PRP) supplementation in semen extender on conception/ pregnancy rate (%) of post-thaw buffalo spermatozoa with 95% CI. Mean difference (MD), standard deviation (SD), and total sample size (Total) of PRP-treated and control groups are presented

evidence of significant asymmetry. However, due to the small number of studies involved, it cannot be confidently stated that publication bias does not exist. Therefore, the effects of small studies might still be present (Fig. 11).

4. Discussion

4.1. General overview of findings

This systematic review and meta-analysis combine experimental studies on the efficacy of platelet-rich plasma (PRP) in semen extender supplements to improve the cryo-survival of buffalo spermatozoa. In all the studies considered here, PRP showed positive effects on important post-thaw sperm parameters such as progressive motility, viability, plasma membrane integrity, and acrosome integrity (Güngör et al. 2025; Hosseini et al. 2025; Moradian et al. 2025). These changes were also associated with a beneficial shift in the oxidative stress markers, mainly the lowering of lipid peroxidation (measured by MDA levels) and boost of antioxidant indicators like TAC and SOD (Tognoloni et al. 2023). Notably, a number of studies, as well as their *in vivo* validation, have shown that after artificial insemination with frozen-thawed semen supplemented with PRP, the conception/ pregnancy rates increased (Liu et al. 2022; Segabinazzi et al. 2017). The set of results are consistent with the notion of PRP as a biological active compound mixture highly enriched in growth factors, cytokines, peptides, and antioxidants. It is known that these bioactive molecules regulate sperm cell survival pathways, keep sperm membranes intact,

up-regulate mitochondrial activities, and down-regulate ROS (Amable et al. 2013; Masuki et al. 2016), which altogether are essential processes involved in sperm preservation when exposed to cryogenic stress.

Even if the designs of PRP-related experiments differ in terms of PRP doses, types of semen extenders, donor fertility status, and yields of platelets, in most cases, the evidence indicates enhanced cryosurvival of sperm cells and reproductive potential when supplemented with PRP (Salama et al. 2024a; Yan et al. 2021). The results connect well with the ongoing agreement in reproductive biotechnology that biologically derived supplements having multidimensional activities like PRP may be better than the use of individual antioxidants or synthetic cryoprotectants solely, especially in buffalo, where due to the high sensitivity of semen to cooling and freezing stresses, this is very relevant. Besides, the findings serve as a Timothy gap filler in the knowledge as no previous summary work has evaluated the size of PRP-induced changes in buffalo semen quality and fertility outcomes.

4.2. PRP and post-thaw sperm quality

Improved motility, viability, stability of membranes, and integrity of the sperm acrosome after thawing are some of the few signs that PRP may be carrying out different cryoprotective roles during the stages of cooling, freezing, and thawing. Sperm motility and viability are very dependent on the fluidity of membranes, the production of energy by mitochondria, and the osmotic condition of the cell. Groups treated with PRP at different doses (5-15%) were found to be better than control

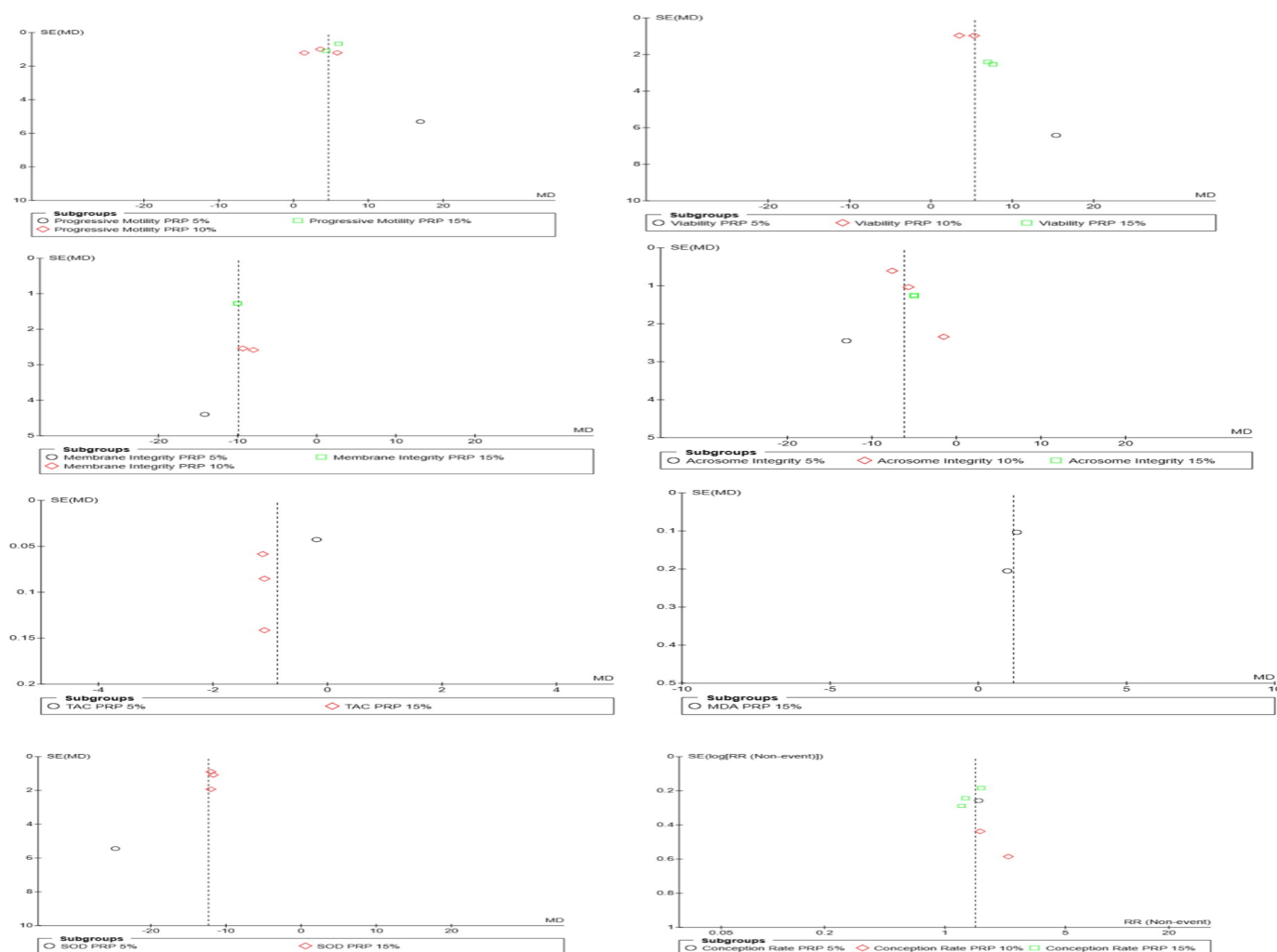


Fig. 10. Funnel plots assessing potential publication bias across all outcome domains included in the meta-analysis, comprising sperm quality parameters (progressive motility, viability, plasma membrane integrity, acrosome integrity), antioxidant biomarkers (MDA, TAC, SOD), and fertility outcomes (conception rate). Each subplot represents the distribution of effect sizes against their standard errors for the respective outcome. Visual symmetry was inspected to evaluate the likelihood of small-study effects or selective reporting across studies

groups in overall motility and viability parameters through meta-analysis, which promotes the hypothesis that PRP helps in membrane stabilization and supports sperm metabolism providing energy for the post-thawing function (Almadaly et al. 2024; Almadaly et al. 2023; El-Sherbiny et al. 2022; Salama et al. 2024a). As a source of precursor-derived proteins, PRP contains platelet growth factor (PDGF), transforming growth factor- β (TGF- β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF-1) (Cecerska-Heryć et al. 2022). These growth factors work by binding to sperm membrane receptors and triggering intracellular signaling cascades to sustain mitochondrial membrane potential, regulate calcium ion influx, and prevent premature capacitation-like events (Wu et al. 2025). In addition, Besides this, fibrinogen- and plasma protein-rich environment of PRP may replicate the protective actions performed by seminal plasma proteins, thus helping to stabilize sperm glycocalyx and protect the plasma membrane and the acrosomal membrane from cold shock.

Earlier works on other animals have also illustrated the beneficial effects of PRP in improving motility, mitochondrial energy production, and sperm DNA integrity after cryopreservation (El-Sherbiny et al. 2022). Also, increased membrane integrity and lessened apoptotic

signaling have been found in rams and bucks (Salama et al. 2024a). In human ART, PRP has also been shown to enhance sperm motility and DNA integrity in sub-fertile men, demonstrating a possibility of its protective mechanisms being broadly conserved across mammalian species (Moradian et al. 2025). Due to the fact that the acrosome is highly susceptible to cryodamage in buffaloes, the overall improved acrosomal integrity through this review gains extra significance. PRP by stabilizing phospholipid bilayers and neutralizing the acrosomal enzymes such as acrosin through lessening the oxidative injury may be slowing down the loss of acrosomal membrane and enzymatic activities during cryopreservation.

4.3. Oxidative stress and antioxidant responses

Cryopreservation leads to an overproduction of reactive oxygen species (ROS), resulting in lipid peroxidation, protein oxidation, mitochondrial damage, and DNA fragmentation (Gualtieri et al. 2021). The strong antioxidant ability of PRP was a clear point from the various experiments reviewed, where PRP supplementation lowered MDA while it improved the levels of TAC and SOD (Moradian et al. 2025). These results support the notion that in cryobiology, the use of multicomponent antioxidant systems can be more effective than that of

single antioxidant compounds. The content of PRP entails both enzymatic and non-enzymatic antioxidant elements. Apart from the fact that platelet degranulation releases antioxidant peptides that can directly neutralize ROS, certain growth factors like IGF-1 and VEGF may indirectly enhance mitochondrial efficiency, and thus decrease ROS generation at its source (Tognoloni et al. 2023).

In a similar fashion, research with sheep sperm samples demonstrated that PRP lessens ROS production and maintains mitochondria membrane potential, which is crucial for sperm to produce the ATP necessary for motility (Güngör et al. 2025). PRP has been shown time and again to improve redox balance and help cells recover faster from oxidative stress in human cell lines and regenerative medicine models. This evidence from various fields supports the idea that the antioxidant work done by PRP is strong and not limited to just one species (Yan et al. 2021). The action of PRP is especially significant in buffalo sperm that usually have lower intracellular antioxidant levels than cattle. The high amount of polyunsaturated fatty acids (PUFA) in buffalo sperm membranes makes them very vulnerable to rapid lipid peroxidation, so the decrease in MDA levels found in this meta-analysis is of biological importance (El-Sherbiny et al. 2022). Further increases in TAC and SOD point to the idea that PRP not only acts through direct ROS scavenging but also enhances the body's own antioxidant defense systems (Li et al. 2021).

4.4. Fertility outcomes (in vivo evidence)

Adding in vivo fertility data is one of the main reasons why this meta-analysis is of high quality. According to the pooled risk ratio (RR), buffaloes receiving PRP-treated frozen-thawed semen were more fertile than those receiving control semen, especially at PRP levels of 10% and 15%. This means that the changes in sperm motility, membrane stability, acrosome integrity, and oxidative balance are linked with real reproductive benefits in sperm transport, selection in the oviduct, capacitation, and fertilization processes. Buffalo reproductive research has shown that fertility can be reliably predicted by post-thaw motility and intact plasma membrane integrity. The review of the buffalo reproduction literature indicates that conception rates at an acceptable level in most cases are associated with post-thaw motility of more than 35–40% and plasma membrane integrity of more than 50%. The results of the present research show that PRP supplementation helps to meet these values, which is a good reason to consider PRP in artificial insemination practices (Almadaly et al. 2023; El-Sherbiny et al. 2022). Functionally, PRP increases motility as well as membrane stability, which extends sperm life in the female reproductive tract and raises the chance of a successful meeting with the oocyte. In addition, PRP might prevent premature acrosome reaction - a major problem in cryopreserved buffalo semen (Chang et al. 2015; Nagy et al. 2023; Seckin et al. 2022). By inhibiting oxidative damage, PRP also helps preserve the DNA, thus supporting the initial stages of embryo development and normal conceptus formation in cows. Fertility improvements related to PRP-like antioxidants have been documented in animals other than buffaloes, e.g. cattle, sheep, and goats (Nazari et al. 2019; Güngör et al. 2025). In buffalo production systems, there is often a serious problem with reproductive efficiency, so even small improvements in pregnancy rates can greatly affect the economics of such systems. Thus, the rationale for PRP combined with actual fertility data makes it a very effective means of improving AI results in buffalo breeding programs.

4.5. Sources of heterogeneity

The quantitative synthesis showed that the effects of PRP were broadly consistent across studies. However, a few methodological and biological factors led to differences in certain parameters. Firstly, differences in PRP concentration between studies (5–15%) potentially cause different dose-response effects. Moderate to high concentrations usually result in better improvement of sperm motility and fertility. Platelet yield and activation status also depend on the PRP preparation method (single vs double centrifugation protocols), which finally determines the concentration of growth factors and bioactive molecules. Another major factor accounting for differences among extenders is the composition of the semen extender. For example, membrane-interactive properties of Tris-egg yolk-, skim milk-, or soybean lecithin-containing extenders may differ and thereby their synergy or competition with PRP components. Another contributor to between-study variability could be donor-related factors. Semen from buffalo bulls of different fertility (fertile vs. sub-fertile) may respond differently to cryopreservation and hence present different levels of improvement after PRP addition. Cryopreservation methods are different from each other in many aspects such as cooling rates, time of equilibration, concentration of glycerol, and temperature of thawing. These are almost always different between labs and can affect sperm survival independently. Besides these, research laboratories differ in the sperm parameter assessment techniques they use (e.g., eosin-nigrosin staining vs. viability assays using fluorescence, manual motility evaluation vs. CASA). This may result in analytical variability among studies. Subgroup analyses in this meta-analysis found little statistical heterogeneity for several outcomes. Nevertheless, the existence of biological and methodological diversity must call attention to the standardization of protocols in PRP cryopreservation research.

4.6. Practical implications for buffalo reproduction

The results of this systematic review and meta-analysis provide a strong foundation for integrating PRP supplementation into buffalo semen cryopreservation practices. PRP represents a biologically active, species-compatible, and relatively low-cost additive that can be produced from the bull itself or from donor animals within breeding stations. For artificial insemination centers and buffalo breeding programs, the application of PRP may increase post-thaw semen usability, reduce straw wastage, and improve conception rates under field conditions. Given the reproductive challenges frequently encountered in buffalo production systems, including seasonal anestrus, lower ovarian responsiveness, and generally lower AI success compared with cattle, PRP supplementation may represent a valuable technological approach to enhance reproductive efficiency and genetic dissemination. Scalability is feasible because PRP preparation requires only standard laboratory centrifugation equipment. However, successful field implementation will require protocol standardization, particularly regarding platelet concentration targets, activation methods, and optimal dilution ratios within semen extenders. Cost-benefit considerations also favor PRP adoption. Compared with synthetic antioxidant additives, PRP can be produced locally at relatively low cost and provides a broader spectrum of protective bioactive molecules. Its multimodal protective mechanisms may therefore offer practical advantages for improving semen cryopreservation outcomes in buffalo artificial insemination programs.

4.7 Future research directions

Despite the positive results of this meta-analysis, several gaps in the research still exist and future research should be directed towards them.

The release of standardized protocols for PRP preparation and characterization should be considered a top priority. Differences in platelet concentration, activation procedures, and processing methods could significantly affect PRP bioactivity. Therefore, setting a benchmark for quality control and determining the optimal platelet concentration ranges would not only help increase reproducibility but also facilitate comparability between different laboratories. Additionally, more research is indispensable to validate the reproductive advantages of PRP administration by using the buffalo gametes under the real-world production conditions that are suitable for the farmers to operate in. Firstly, there are very few *in vivo* studies that have been done yet, while there might be other improved semen quality and fertility parameters but that information is still limited. So, a large number of artificial insemination trials carried out in different buffalo populations and production systems will give more reliable evidence on the practicality of PRP use in breeding programs.

It is very necessary to research the level of PRP in artificial insemination semen extenders most suitable for human use. High concentrations of platelets may adversely affect the sperm's physiology coexisting with cytokine imbalance due to hyperactive levels of platelets, however, a sufficient concentration of platelets seems to be effective in the sperm cryosurvival process which necessitates well-planned PRP dose-response investigations. Additionally, biosafety issues also need to be considered especially the danger of microbial contamination of the PRP during its production which drives the need for standardized aseptic procedures in artificial insemination labs for PRP safe use. Besides, it would be great if future studies can monitor the long-term effect of PRP-treated semen on reproduction such as the development of the embryo, the health of the offspring, and even the possible epigenetic changes so that we can understand more about the extensive biological effects of PRP supplementation.

5. Conclusions

This systematic review and meta-analysis reveals that the supplementation of platelet-rich plasma (PRP) during cryopreservation of buffalo sperm improves the post-thaw quality and fertility potential. The PRP supplementation significantly boosts the sperm functions such as progressive motility, cell viability, plasma membrane stability, and acrosome integrity, while limiting lipid peroxidation and increasing the sperm's natural antioxidant system. These sperm *in vitro* improvements result in increased pregnancy rates, confirming the practical biological advantages of PRP. The growth factors, antioxidants, and particular plasma proteins released after the activation of platelets collectively alleviate the cryo-induced structural and metabolic issues. These ingredients potentially ensure the normal functioning of sperm mitochondria, and help regulate membrane structure and function after thawing. Most of the inconsistencies observed in this meta-analysis could be easily accounted for by the differences in PRP concentration, method of preparation, extender systems used, or the reproductive status of semen donors rather than by non-conforming biological responses.

Declarations

Funding: This study received no external funding

Conflict of interest: The authors declare that there are no conflicts of interest related to the design, execution, analysis, or publication of this systematic review and meta-analysis

Acknowledgements: The authors express appreciation for publicly accessible databases that enabled completion of this work

Ethics approval: Ethical approval was not required because this study was a systematic review and meta-analysis of previously published data and did not involve experimental procedures on live animals

Declaration of AI use: The authors used Rayyan AI (Rayyan Systems Inc., Qatar; Version 2024) to support the screening and organization of literature during the study selection process, including title/abstract screening and duplicate detection. ChatGPT (OpenAI, USA; GPT-5.1 model, 2025 version) was used exclusively to enhance the linguistic clarity and readability of the manuscript. All research concepts, methodological decisions, data interpretation, meta-analytic judgments, and intellectual content including framing of results, discussion, and conclusions were entirely generated by the authors without AI involvement. The authors maintained full control over the analytical and scientific reasoning throughout the study.

References

- Abdulla AK, Rebai T, Al-Delemi DHJ. (2022). Protective effects of autologous Platelet-Rich Plasma (PRP) on the outcome of cryopreservation in rabbit sperm. *Cellular and Molecular Biology* 68(4): 113–121. <https://doi.org/10.14715/cmb/2022.68.4.14>
- Alcay S, Aktar A, Koca D, Kilic MA, Akkasoglu M, Yilmaz MM, Sagirkaya H. (2022). Autologous platelet rich plasma have positive effect on ram spermatozoa during cryopreservation in non-breeding season. *Kafkas Universitesi Veteriner Fakultesi Dergisi* 28(2): 229-234. <https://doi.org/10.9775/kvfd.2021.26763>
- Almadaly E, Mansour I, Sahwan F, Shehabeldin MA, El-Kon I, Sakr A, Eldomany W, Ramoun AA. (2024). 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. *Egyptian Journal of Veterinary Sciences* 56(3): 617–626. <https://doi.org/10.21608/ejvs.2024.254249.1712>
- Almadaly EA, Ibrahim IM, Salama MS, Ashour MA, Sahwan FM, El-Kon II, Abouzed TK, El-Domany WB. (2023). Effect of platelet-rich plasma (PRP) on post-thaw quality, kinematics and *in vivo* fertility of fertile and subfertile buffalo (*Bubalus bubalis*) spermatozoa. *Veterinary Research Communications* 47(1): 61–72. <https://doi.org/10.1007/s11259-022-09928-1>
- Amable PR, Carias RBV, Teixeira MVT, Da Cruz Pacheco Í, Corrêa Do Amaral RJE, Granjeiro JM, Borojevic R. (2013). Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. *Stem Cell Research & Therapy* 4(3): 67. <https://doi.org/10.1186/srct218>
- Bustani GS, Baiee FH. (2021). Semen extenders: An evaluative overview of preservative mechanisms of semen and semen extenders. *Veterinary World* 14(5): 1220–1233. <https://doi.org/10.14202/vetworld.2021.1220-1233>
- Cecerska-Heryć E, Goszka M, Serwin N, Roszak M, Grygorcewicz B, Heryć R, Dołęgowska B. (2022). Applications of the regenerative capacity of platelets in modern medicine. *Cytokine & Growth Factor Reviews* 64(2022): 84–94. <https://doi.org/10.1016/j.cytogfr.2021.11.003>
- Chang Y, Li J, Chen Y, Wei L, Yang X, Shi Y, Liang X. (2015). Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during *in vitro* fertilization. *International journal of clinical and experimental medicine* 8(1): 1286–1290. <https://pmc.ncbi.nlm.nih.gov/articles/PMC4358582/>
- El-Sherbiny HR, Abdelnaby EA, Samir H, Fathi M. (2022). Addition of autologous platelet rich plasma to semen extender enhances cryotolerance and fertilizing capacity of buffalo bull spermatozoa. *Theriogenology* 194: 104–109. <https://doi.org/10.1016/j.theriogenology.2022.09.029>
- Ezzati M, Shانهbandi D, Hamdi K, Rahbar S, Pashaiasl M. (2020). Influence of cryopreservation on structure and function of mammalian spermatozoa: an overview. *Cell Tissue Bank* 21(1): 1–15. <https://doi.org/10.1007/s10561-019-09797-0>

- Gualtieri R, Kalthur G, Barbato V, Di Nardo M, Adiga SK, Talevi R. (2021). Mitochondrial dysfunction and oxidative stress caused by cryopreservation in reproductive cells. *Antioxidants* 10(3): 337. <https://doi.org/10.3390/antiox10030337>
- Güngör İH, Türk G, Çakır Cihangiroğlu A, Arkalı G, Tektemur A, Kırmızıyaka Özmen G, Badıllı N, Acısu TC, Bulan MS, Özer Kaya Ş, Sönmez M, Gür S, Yüce A, Yılmaz Ö, Memili E, Çambay Z. (2025). Platelet-rich plasma strategy against freezing damage in ram spermatozoa: its effect on miRNA, ion channels, growth factors, lipids and oxidative stress. *Reproduction, Fertility and Development* 37(15): RD25064. <https://doi.org/10.1071/RD25064>
- Hosseini S, Kazemi M, Salehpour S, Saharkhiz N, Majidi L. (2025). Evaluation of the effect of platelet-rich plasma (PRP) on the sperm parameters. *Taiwanese Journal of Obstetrics and Gynecology* 64(2): 313–318. <https://doi.org/10.1016/j.tjog.2024.12.010>
- Hufana-Duran D, Chaikhun-Marcou T, Duran PG, Atabay EP, Nguyen HT, Atabay EC, Nguyen UT, Nguyen HT, Hiew MWH, Punyawai K, Ginting N, Parmpai R. (2025). Future of reproductive biotechnologies in water buffalo in southeast Asian countries. *Theriogenology* 233: 123–130. <https://doi.org/10.1016/j.theriogenology.2024.11.016>
- Khan IM, Cao Z, Liu H, Khan A, Rahman SU, Khan MZ, Sathanawongs A, Zhang Y. (2021). Impact of cryopreservation on spermatozoa freeze-thawed traits and relevance OMICS to assess sperm cryo-tolerance in farm animals. *Frontiers in Veterinary Science* 8: 609180. <https://doi.org/10.3389/fvets.2021.609180>
- Li WH, Weng XX, Yuan LF, Li F, Yue XP, Li FD. (2021). Effect of feeding linseed diet on testis development, antioxidant capacity, and epididymal cauda sperm concentration in Chinese Hu lamb. *Theriogenology* 159: 69–76. <https://doi.org/10.1016/j.theriogenology.2020.10.014>
- Liu K, Cheng H, Guo Y, Liu Y, Li L, Zhang X. (2022). Autologous platelet-rich plasma intrauterine perfusion to improve pregnancy outcomes after implantation failure: A systematic review and meta-analysis. *Journal of Obstetrics and Gynaecology Research* 48(12): 3137–3151. <https://doi.org/10.1111/jog.15431>
- Masuki H, Okudera T, Watanebe T, Suzuki M, Nishiyama K, Okudera H, Nakata K, Uematsu K, Su C-Y, Kawase T. (2016). Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF). *International Journal of Implant Dentistry* 2(1): 19. <https://doi.org/10.1186/s40729-016-0052-4>
- Moradian SA, Amirkhani Z, Movahedin M, Gharib SN, Varghaiyan Y, Niknafs B, Hamdi K, Hajipour H, Fattahi A. (2025). Platelet-rich plasma (PRP) and the future of male fertility: a path forward for personalized and regenerative therapies. *Stem Cell Research & Therapy* 16(1): 486. <https://doi.org/10.1186/s13287-025-04602-0>
- Nagy B, Kovács K, Sulyok E, Várnagy Á, Bódis J. (2023). Thrombocytes and platelet-rich plasma as modulators of reproduction and fertility. *International Journal of Molecular Sciences* 24(24): 17336. <https://doi.org/10.3390/ijms242417336>
- Nazari A, Dirandeh E, Ansari-Pirsaraei Z, Deldar H. (2019). Antioxidant levels, copper and zinc concentrations were associated with postpartum luteal activity, pregnancy loss and pregnancy status in Holstein dairy cows. *Theriogenology* 133: 97–103. <https://doi.org/10.1016/j.theriogenology.2019.04.034>
- Partyka A, Nizański W. (2021). Supplementation of avian semen extenders with antioxidants to improve semen quality—Is it an effective strategy?. *Antioxidants* 10(12): 1927. <https://doi.org/10.3390/antiox10121927>
- Pavlovic V, Ciric M, Jovanovic V, Stojanovic P. (2016). Platelet Rich Plasma: a short overview of certain bioactive components. *Open Medicine* 11(1): 242–247. <https://doi.org/10.1515/med-2016-0048>
- Salama MS, Shehabeldin AM, Ashour MA, Al-Ghadi MQ, Marghani BH, El-kon I, Shukry M. (2024a.) Effect of the addition of platelet-rich plasma to Boer buck semen on sperm quality and antioxidant activity before and after cryopreservation and in vivo fertility. *Small Ruminant Research* 230: 107167. <https://doi.org/10.1016/j.smallrumres.2023.107167>
- Salama MS, Shehabeldin AM, Ashour MA, Alkeridis LA, Al-Shuraym LA, Sayed SM, Farrag F, Shukry M. (2024b). The impact of autologous platelet-rich plasma supplement on Ossimi ram semen quality and fertility: In vivo fertility of freshly diluted and frozen-thawed spermatozoa. *Small Ruminant Research* 235: 107283. <https://doi.org/10.1016/j.smallrumres.2024.107283>
- Seckin S, Ramadan H, Mouanness M, Kohansieh M, Merhi Z. (2022). Ovarian response to intraovarian platelet-rich plasma (PRP) administration: hypotheses and potential mechanisms of action. *Journal of Assisted Reproduction and Genetics* 39(1): 37–61. <https://doi.org/10.1007/s10815-021-02385-w>
- Segabinazzi LG, Friso AM, Correal SB, Crespilho AM, Dell'Aqua JA, Miró J, Papa FO, Alvarenga MA. (2017). Uterine clinical findings, fertility rate, leucocyte migration, and COX-2 protein levels in the endometrial tissue of susceptible mares treated with platelet-rich plasma before and after AI. *Theriogenology* 104: 120–126. <https://doi.org/10.1016/j.theriogenology.2017.08.007>
- Tognoloni A, Bartolini D, Pepe M, Di Meo A, Porcellato I, Guidoni K, Galli E, Chiaradia E. (2023). Platelets rich plasma increases antioxidant defenses of tenocytes via Nrf2 signal pathway. *International Journal of Molecular Sciences* 24(17): 13299. <https://doi.org/10.3390/ijms241713299>
- Ullah K, Waheed A. (2025). Review: Genetic improvement in livestock: A journey from conventional breeding to genomic precision. *Veterinary Biomedical and Clinical Journal* 7(1): 30–44. <https://doi.org/10.21776/ub.VetBioClinJ.2025.007.01.3>
- Wu W-S, Chen L-R, Chen K-H. (2025). Platelet-Rich Plasma (PRP): Molecular mechanisms, actions and clinical applications in human body. *International Journal of Molecular Sciences* 26(21): 10804. <https://doi.org/10.3390/ijms262110804>
- Yan B, Zhang Y, Tian S, Hu R, Wu B. (2021). Effect of autologous platelet-rich plasma on human sperm quality during cryopreservation. *Cryobiology* 98: 12–16. <https://doi.org/10.1016/j.cryobiol.2021.01.009>

Citation

Prastiya RA, Sardjito T, Mayawati SP, Purnama MTE, Saputro AL, Agustono B, Haryuni N, Sasi SM, Alghoul NM. (2026). Platelet-rich plasma supplementation in semen extenders improves post-thaw sperm quality and fertility in buffalo (*Bubalus bubalis*): A systematic review and meta-analysis. *Letters in Animal Biology* 06(1): 46 – 59. <https://doi.org/10.62310/liab.v6i1.303>