

Potential role of platelet rich plasma (PRP) to improve bovine semen quality: A review

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ABSTRACT

Platelet-Rich Plasma (PRP) is a biological innovation increasingly being researched in animal reproduction due to its content of various growth factors, cytokines, and bioactive components that support cellular activity. In male reproduction, the use of PRP has begun to attract attention due to its ability to improve bovine semen quality, particularly in artificial insemination and cryopreservation procedures that often reduce sperm motility, viability, and membrane integrity. This review aimed to compile an up-to-date summary of the mechanism of action of PRP in maintaining and improving bovine semen quality while also examining published experimental findings. Physiologically, PRP releases growth factors, including PDGF, TGF- β , IGF, and VEGF, which play a key role in cell regeneration, membrane stability, and reducing oxidative stress. This process is believed to create a more supportive microenvironment for spermatozoa, increase their resistance to damage during storage and freezing, and improve semen parameters such as progressive motility, plasma membrane integrity, and fertilization ability. Although most studies report positive results, differences in PRP preparation techniques, concentrations, and application methods still contribute to variations in research results. Therefore, this review emphasizes the need for standardized protocols and further studies to ensure the effectiveness and safety of PRP use in bovine reproductive practices. With more targeted methods, PRP has the potential to become a new biological agent that can improve reproductive performance and the efficiency of insemination programs.

Introduction

Semen quality is a key component in the success of cattle reproductive programs, particularly in modern reproductive systems that rely heavily on artificial insemination (AI) (Tanga *et al.*, 2021). Decreased semen quality is characterized by reduced motility, viability, membrane integrity, and increased DNA damage in sperm has become a significant issue in the livestock industry (Kudratullah *et al.*, 2024). This problem not only reduces pregnancy rates but also reduces production efficiency and lengthens the intercalation interval, ultimately impacting livestock productivity and profitability (Handarini *et al.*, 2024). Several factors, including heat stress, inadequate nutritional management, reproductive disorders, bull age, and genetic variation, are known to contribute to semen quality degradation (Capela *et al.*, 2022). Therefore, innovative approaches are needed to maintain optimal male reproductive function.

In artificial insemination, one of the biggest challenges is maintaining the quality of frozen semen after cryopreservation (Spanner *et al.*, 2025). Although semen freezing technology has long been used to distribute superior genetic material, this procedure can naturally cause structural and functional damage to spermatozoa (Nagata *et al.*, 2019). Factors such as exposure to extreme temperatures, ice crystal formation, changes in osmotic pressure, and increased oxidative stress contribute to the decline in semen quality after thawing (Deng *et al.*, 2025). These conditions often result in lower fertility rates in frozen semen compared to fresh semen, raising the need for more effective protective approaches to improve spermatozoa survival during freezing (Qin *et al.*, 2025). Various additives such as antioxidants, amino acids, and bioactive compounds have been

tested, but the results are still mixed and not yet fully adequate to meet the needs of field applications (Shahidi and Samarasinghe, 2025).

Platelet-Rich Plasma (PRP) is now seen as a biological innovation that has the potential to offer solutions to these various problems (Wu *et al.*, 2025). PRP is a plasma fraction with a significantly higher platelet concentration than physiological levels and contains various growth factors, cytokines, chemokines, and other bioactive molecules that function in cell regeneration and protection (Zhang *et al.*, 2025). In human medical practice, PRP has been widely used to accelerate tissue repair, suppress inflammation, and stimulate cell regeneration (Xu *et al.*, 2020). Research advances also indicate that PRP is beginning to be applied in the reproductive field, including for ovarian therapy, endometrial repair, and improving germ cell quality (Karadbhajne *et al.*, 2024). The bioactive components contained in PRP are thought to influence the microenvironment of reproductive cells, reducing oxidative stress, and increasing membrane stability mechanisms that are crucial for maintaining spermatozoa quality (Moustakli *et al.*, 2025).

However, knowledge regarding the effects of PRP on bovine semen remains limited and lacks clear standards (Caterino *et al.*, 2023). Most available research is preliminary and exploratory, with differences in PRP preparation techniques, concentration variations, and application procedures yielding inconsistent findings (Bacevich *et al.*, 2024). Furthermore, the molecular mechanisms of PRP interaction with spermatozoa remain incompletely elucidated (Anifandis *et al.*, 2014). This situation indicates a significant knowledge gap regarding the potential, limitations, and safety aspects of PRP use in bovine reproductive biotechnology (Moradian *et al.*, 2025). More comprehensive research is needed to confirm whether PRP is

truly capable of providing consistent benefits in improving semen quality, both in fresh and frozen semen (Hosseini *et al.*, 2025).

With these considerations in mind, this review aimed to summarize recent developments regarding the use of PRP in improving bovine semen quality, examine the underlying biological mechanisms, and identify limitations of existing research. Furthermore, this paper aimed to provide direction for future research. Overall, this manuscript is expected to serve as a comprehensive scientific reference for researchers, reproductive practitioners, and livestock industry players in understanding the potential of PRP as an innovative biological agent for optimizing bovine semen quality.

Basic concept of platelet rich plasma

PRP has emerged as one of the most promising biological technologies in regenerative medicine and animal reproduction (Moustakli *et al.*, 2025). Its effectiveness is supported by its ability to provide high concentrations of platelets along with various bioactive components that play a role in cell repair, inflammation regulation, and tissue function (Zhang *et al.*, 2019). To assess PRP's potential for improving bovine semen quality, a more comprehensive understanding of its definition, working principles, and the variety of preparation methods that can influence its biological activity is required.

Definition and characteristics of PRP

PRP is a plasma component containing a significantly higher platelet count than the normal concentration in whole blood (Everts *et al.*, 2020). This product is obtained by separating blood components using a centrifugation method, which allows for an increase in platelet count without compromising their structural integrity or function (Chicharro-Alcántara *et al.*, 2018). PRP typically contains approximately 2 to 5 times more platelets than normal plasma, although this can vary depending on the preparation procedure and the centrifugation force used (Alves and Grimalt, 2018). As a biological product, PRP has a unique composition that distinguishes it from ordinary plasma and other blood derivatives (Patel *et al.*, 2023).

Structurally, PRP is enriched with various bioactive components released from alpha granules and high-density granules in platelets (Pavlovic *et al.*, 2016). These components include several important growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF- β), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), and fibroblast growth factor (FGF) (Pineda-Cortel *et al.*, 2024). These factors play a role in triggering cell proliferation and differentiation, supporting angiogenesis, and aiding tissue recovery (Verma *et al.*, 2023). Furthermore, PRP also contains cytokines, chemokines, adhesion proteins, and antioxidant molecules that can reduce free radicals and provide protection against oxidative damage to cells (Hudgens *et al.*, 2016).

The high concentration of platelets in PRP makes it a bioactive source that can interact with various cell types, including spermatozoa (Hosseini *et al.*, 2025). In addition to growth factors, PRP also contains several plasma proteins such as fibrinogen, fibronectin, and vitronectin, which contribute to tissue healing and help maintain cell membrane stability (Wang *et al.*, 2025a). However, PRP characteristics can vary significantly between preparations containing leukocytes (leukocyte-rich PRP) and preparations that have been depleted of leukocytes (leukocyte-poor PRP) (Tey *et al.*, 2022). These differences affect the inflammatory response, cytokine levels, and molecular properties of PRP when applied to biological systems (Wu *et al.*, 2025).

How PRP works

PRP functions through a series of complex biological mechanisms,

primarily by releasing various growth factors, cytokines, and active biomolecules that play a role in regulating cell activity (Imam *et al.*, 2022). The concentrated platelets in PRP contain alpha granules, the primary source of growth factors such as PDGF, TGF- β , VEGF, IGF-1, and EGF (Cecerska-Heryć *et al.*, 2022). Each of these growth factors has a unique target and mode of action, which collectively supports improved cell function, tissue regeneration, and maintains the stability of the surrounding microenvironment (Altalbawy *et al.*, 2025).

PDGF functions to stimulate the proliferation and migration of various cell types, including epithelial cells and fibroblasts, which play a crucial role in tissue regeneration (Irma *et al.*, 2025). TGF- β acts as a key regulator of cell differentiation and local immune responses and is involved in tissue remodeling (Massagué and Sheppard, 2023). VEGF plays a role in triggering the formation of new blood vessels and increasing blood flow to tissues, thereby helping supply oxygen and nutrients to cells (Mahaki *et al.*, 2025). IGF-1, present in high levels in PRP, supports cellular metabolic activity, including mitochondrial function, which influences sperm motility and survival (Shin *et al.*, 2014). Meanwhile, EGF contributes to epithelial cell proliferation and repair, and helps maintain cell membrane stability (Tito *et al.*, 2025).

In addition to containing various growth factors, PRP is also enriched with cytokines and other biomolecules, including interleukins, chemokines, and antioxidant compounds that play a role in regulating the inflammatory process (Huber *et al.*, 2021). The anti-inflammatory compounds in PRP can reduce the formation of free radicals and minimize oxidative damage that can affect cell membranes and genetic material (Nakadate *et al.*, 2025). Furthermore, the presence of biomolecules such as fibrinogen, fibronectin, and vitronectin also supports the stability of cell structure and enhances the ability of cells to adhere to and interact with each other (Parisi *et al.*, 2020).

PRP preparation variations

Differences in PRP preparation methods are important factors affecting platelet count, cell composition, and the diversity of bioactive molecules within the PRP (Andia *et al.*, 2025). Broadly speaking, PRP is classified based on its leukocyte content into leukocyte-poor PRP (LP-PRP) and leukocyte-rich PRP (LR-PRP) (Lana *et al.*, 2019). LP-PRP has a very low leukocyte concentration, making it more suitable for use in conditions requiring anti-inflammatory and regenerative effects without triggering an excessive inflammatory response (Lu *et al.*, 2023). Conversely, LR-PRP contains a higher number of leukocytes, providing stronger immunomodulatory potential, but may increase the risk of local inflammation in sensitive tissues (Tian *et al.*, 2025). Therefore, the choice of PRP type must be tailored to the biological objectives and physiological conditions of the target tissue (Santos *et al.*, 2024).

The PRP production procedure significantly impacts the quality of the final product (Abu-Ghname *et al.*, 2019). The two most used techniques are single-spin and double-spin centrifugation. In the single-spin method, platelet-rich plasma is separated from other blood components through a single centrifugation process (Harrison *et al.*, 2023). This approach is more practical, faster, and reduces the risk of platelet damage, although the resulting platelet concentration tends to be lower (Harrison *et al.*, 2020). In contrast, the double-spin method involves two centrifugations: one to separate the plasma from the red blood cells, and the other to increase the platelet concentration (Dhurat and Sukesh, 2014). This technique can produce PRP with a higher and more consistent platelet count but carries the risk of premature platelet activation if centrifugation parameters are not properly controlled (Legiawati *et al.*, 2023).

Although various methods are available, one of the main obstacles to PRP production is the lack of an agreed-upon formulation standard (Andia *et al.*, 2025). Variations in centrifugation speed and duration, tube type, temperature, plasma collection technique, and donor blood quality lead to significant differences in the biological composition of PRP

between studies and clinical practice (Zhou *et al.*, 2025). The lack of international guidelines regarding optimal platelet concentration ranges, leukocyte counts, and the most appropriate activation methods further complicates the comparison of research results (Boffa *et al.*, 2024). Therefore, efforts towards standardization are crucial to ensure data consistency, improve therapy efficacy, and encourage broader use of PRP in the reproductive field, including in improving bovine semen quality (Kooli *et al.*, 2025). The biological mechanism by which PRP influences bovine spermatozoa is illustrated in Figure 1, highlighting the key growth factors and antioxidant actions involved in membrane stabilization, oxidative stress reduction, mitochondrial enhancement, and DNA repair support.

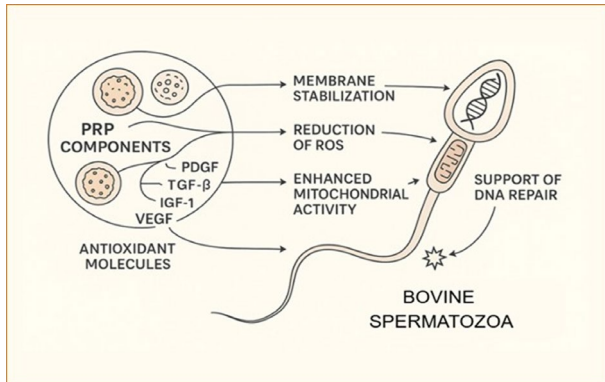


Figure 1. Schematic representation of PRP mechanisms acting on bovine spermatozoa.

Quality of bovine semen and influencing factors

Semen quality in cattle is a key factor in the success of reproductive programs, particularly in artificial insemination and semen freezing (Tanga *et al.*, 2021). Assessing various semen parameters and understanding the biological and environmental factors that influence them are crucial to ensuring maximum fertility (Odinius *et al.*, 2024). Furthermore, the challenges encountered during cryopreservation require innovative strategies to maintain spermatozoa structure and function after thawing.

Semen quality parameters

Evaluating bovine semen quality is a crucial component in assessing a bull's fertility potential and determining the success of an artificial insemination program (Bollwein and Malama, 2023). This assessment involves various biological parameters that indicate the morphological and functional condition of spermatozoa (Silva *et al.*, 2025). One key parameter is motility, the ability of sperm to move progressively toward the egg (Van de Hoek *et al.*, 2022). Good motility reflects optimal mitochondrial performance and intact flagellar structure, making it a key indicator in predicting fertilization potential (Shapovalova *et al.*, 2025).

In addition to motility, sperm viability also plays a crucial role in determining reproductive success (Mangun *et al.*, 2025). Viability indicates the percentage of living sperm with intact plasma membranes, enabling them to maintain osmotic balance, undergo capacitation, and undergo the acrosome reaction normally (Xue *et al.*, 2025). Sperm with membranes damaged by environmental stress or oxidative stress will lose these capabilities and ultimately be unable to fertilize an oocyte (Wang *et al.*, 2025b).

Furthermore, sperm morphology is also an important parameter, including evaluation of the shape of the head, midpiece, and tail (Pelzman and Sandlow, 2024). Morphological abnormalities usually indicate problems with sperm formation or damage that occurs during semen handling (Jakubik-Uljasz *et al.*, 2020). An increased number of abnormally shaped sperm is generally associated with decreased fertilization potential, both in natural reproduction and through artificial insemination techniques (Dursunoglu, 2025).

In addition to structural aspects, the quality of the plasma membrane

and acrosome also plays a crucial role in determining the optimal function of sperm (Simsek *et al.*, 2025). The plasma membrane protects sperm from various physical and chemical damage, while the acrosome plays a crucial role in the penetration of the zona pellucida (Hirose *et al.*, 2020). Disruption of either of these components can inhibit the acrosome reaction and ultimately reduce the chances of fertilization (Kaltsas, 2023).

At the molecular level, sperm DNA quality is now an increasingly researched indicator (Liu *et al.*, 2023). DNA damage, whether in the form of fragmentation or denaturation, can disrupt early embryo development and reduce the chances of pregnancy (Baran *et al.*, 2025). Various factors, such as heat stress, toxin exposure, systemic illness, and even semen freezing (cryopreservation), often contribute to this DNA damage (Marinero and Schlegel, 2023).

Finally, the level of oxidative stress, characterized by the formation of reactive oxygen species (ROS), is an important indicator in evaluating sperm quality (Wang *et al.*, 2025b). While a certain amount of ROS is necessary to support the capacitation process, excess ROS can trigger lipid peroxidation in membranes, damage proteins, and cause DNA fragmentation (Kaltsas, 2023). An imbalance between ROS production and antioxidant capacity is often a major factor in semen quality decline, particularly during storage and cryopreservation (Evans *et al.*, 2021).

Biological factors that affect semen quality

Semen quality in cattle is influenced by various biological factors that contribute directly and indirectly to sperm formation, maturation, and cell stability during storage (Suprayogi *et al.*, 2020). The age of the bull is one of the most important physiological factors (Murphy *et al.*, 2018). Young bulls generally produce less than optimal semen because their reproductive systems are still developing, while aging bulls often experience decreased semen quality due to testicular tissue degeneration, decreased Leydig cell function, and increased incidence of sperm DNA damage (Chao *et al.*, 2023). Optimal reproductive conditions typically occur during the productive adult phase, when hormone production, spermatogenesis activity, and ejaculation ability are at their most optimal levels (Flowers, 2022).

Besides age, nutritional status also significantly determines semen quality (Khan *et al.*, 2024). The availability of macronutrients such as protein, fat, and energy, as well as micronutrients including vitamins A, E, C, selenium, and zinc, is closely linked to testicular health, mitochondrial function, and the body's natural antioxidant activity (Fagbohun *et al.*, 2023). Nutritional deficiencies can disrupt spermatogenesis, increase sperm morphological abnormalities, and exacerbate oxidative stress (Bocu *et al.*, 2024). Conversely, a balanced nutritional intake can increase sperm count and quality by optimizing metabolism in reproductive cells (Skoracka *et al.*, 2020).

The health of the bull also plays a significant role in determining semen quality (Suprayogi *et al.*, 2020). Systemic infections, metabolic disorders, inflammation of the reproductive tract, and hormonal abnormalities can all reduce reproductive capacity (Vannuccini *et al.*, 2016). For example, fever, which causes temporary damage to the testicular epithelium, can lead to decreased semen quality over the following weeks (Akhigbe *et al.*, 2024). Illnesses affecting the immune system can also increase ROS production and damage sperm membranes (Potiris *et al.*, 2025). Therefore, stable bull health is a crucial factor in ensuring semen quality (Gualtieri *et al.*, 2021a).

Another significant biological factor is environmental stress, such as heat exposure, high humidity, and less-than-ideal cage management (Oke *et al.*, 2021). Heat stress is a major cause of semen quality decline in tropical regions (Khan *et al.*, 2023). An increase in scrotal temperature just a few degrees above normal can inhibit spermatogenesis, increase the number of sperm with morphological abnormalities, and accelerate lipid peroxidation in sperm cell membranes (De Toni *et al.*, 2023). Furthermore, stress from transportation, excessive handling, or uncomfortable

cage conditions can trigger the release of stress hormones such as cortisol, which are known to disrupt reproductive hormone production and reduce sperm quality (Ilacqua et al., 2018).

Challenges in semen cryopreservation

Semen cryopreservation is an important method in artificial insemination programs because it allows for long-term storage and widespread distribution of genetic material (Castro et al., 2025). However, the process of freezing and thawing semen still faces several biological challenges that can reduce sperm quality (Ozimic et al., 2023). One major challenge is cold shock, which is cell damage caused by a drastic drop in temperature during the cooling phase (Bartolo-Aguilar et al., 2022). Bovine sperm is highly sensitive to temperature changes due to the unsaturated fatty acids in its membrane, which are easily disrupted by extreme temperature changes (Capela et al., 2022). Cold shock can disrupt the fluidity of the plasma membrane, thus affecting the physiological function of sperm (Castro et al., 2025).

In addition to the effects of cold shock, the cryopreservation process also triggers a significant increase in ROS production (Kuželová et al., 2024). During freezing, changes in osmotic pressure and damage to mitochondria cause ROS to form beyond physiological limits (Zhang et al., 2024). This accumulation of ROS then leads to lipid peroxidation in the membrane, protein oxidation, and damage to sperm DNA (Gualtieri et al., 2021b). This high level of oxidative stress is a major factor in reducing sperm viability and motility after thawing (Sengupta et al., 2024).

Damage to cell membrane structures is also a major issue in cryopreservation (Jaiswal and Vagga, 2022). During the freezing process, the formation of ice crystals both inside and outside the cells can cause membrane rupture, acrosome damage, and ionic imbalance (Huang et al., 2025). Sperm with damaged membranes lose their ability to maintain cellular homeostasis, making them unable to undergo capacitation or fertilization optimally after thawing (Hai et al., 2024).

The accumulated effects of cold shock, oxidative stress, and membrane damage lead to decreased sperm motility after thawing, a key parameter for successful cryopreservation (Castro et al., 2025). Post-thawing semen motility is typically lower than fresh semen due to decreased mitochondrial function, damage to flagellar structure, and impaired energy metabolism (Xu et al., 2025). This decreased motility directly impacts fertilization rates and poses a significant obstacle to the application of cryopreservation on an industrial scale (Raad et al., 2024). The cryopreservation workflow and the associated cellular damage mechanisms during cooling, freezing, and thawing stages are illustrated in Figure 2.

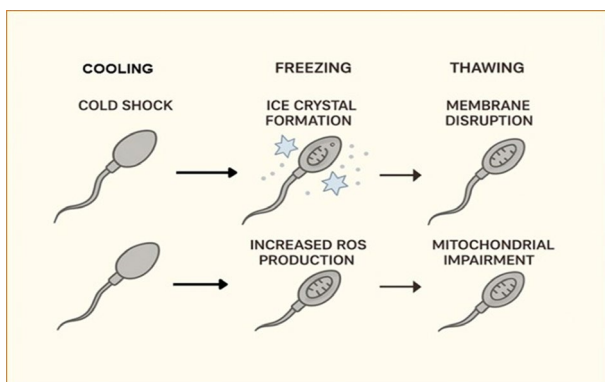


Figure 2. Cryopreservation process and key damage points in bovine spermatozoa.

The potential of PRP to improve the quality of cattle semen

PRP is increasingly recognized as an innovative biological agent with the potential to support the reproductive quality of bulls (Caterino et al., 2023). Thanks to its content of growth factors, cytokines, and various

other bioactive molecules, PRP can provide cellular protection, improve spermatozoa function, and enhance the success of various reproductive biotechnology techniques (Demyashkin et al., 2025). A thorough understanding of PRP's mechanism of action and its application both in vitro and in vivo provides an essential foundation for assessing its effectiveness in improving bovine semen quality.

Biological mechanism of PRP function on sperm cells

PRP functions as a source of various growth factors, cytokines, and bioactive molecules that can modulate sperm performance through several biological mechanisms (Kooli et al., 2025). One of the main mechanisms is providing protection to the sperm cell membrane (Popov et al., 2023). The plasma membrane contains polyunsaturated fatty acids that are highly susceptible to peroxidation (El-Sherbiny et al., 2022). Growth factors such as PDGF, IGF-1, and TGF- β contained in PRP play a role in increasing membrane stability by promoting structural repair and maintaining lipid integrity (Shang et al., 2025). These effects are important for maintaining sperm motility, fertilization capacity, and survival during storage and cryopreservation (Casati et al., 2014).

Furthermore, PRP also exhibits strong antioxidant activity through the presence of enzymes and bioactive peptides capable of neutralizing ROS (Buenfil-Chi et al., 2025). Although ROS at physiological levels are necessary for the capacitation process, excessive accumulation can trigger oxidative stress that damages sperm membranes, proteins, and DNA (Mo et al., 2025). Components such as platelet factor-4, superoxide dismutase, and glutathione peroxidase contained in PRP work synergistically to reduce oxidative stress levels, thereby improving sperm viability and quality, especially in conditions that increase ROS production, such as metabolic disorders, inflammation, and cryopreservation (Muti et al., 2025).

PRP also contributes to improving metabolism and mitochondrial function, which are central to energy production for sperm motility (Sumbalová et al., 2024). Various growth factors in PRP are known to increase enzyme activity in the electron transport chain and improve mitochondrial structure in the sperm midpiece (Kooli et al., 2025). Optimizing mitochondrial function results in more efficient ATP production, thus supporting increased progressive motility, hyperactive movement, and sperm's ability to penetrate the zona pellucida (Van de Hoek et al., 2024).

Finally, PRP also plays a role in repairing DNA damage and sperm cell membranes (Salama et al., 2024). Bioactive components such as VEGF and IGF-1 activate regenerative pathways that help stabilize chromatin structure and repair DNA damage caused by fragmentation and oxidative stress (Liu et al., 2025). Furthermore, these regenerative factors also accelerate the repair of micro-ruptures in the cell membrane, thereby increasing sperm's resilience to unstable environmental conditions (Wu et al., 2025).

In Vitro PRP application on bovine semen

The in vitro use of PRP in bovine semen has become an innovative approach to improving spermatozoa quality, particularly during storage and cryopreservation (Soltani et al., 2025). One application technique involves adding PRP to a semen extender. The extender maintains sperm viability, and the presence of PRP enriches the formulation with growth factors, cytokines, and antioxidant molecules that work together to protect sperm from various structural and biochemical damage (El-Sherbiny et al., 2022). The integration of PRP into the extender is thought to improve membrane stability, maintain enzyme activity, and prevent the decline in motility that often occurs during low-temperature storage and freezing (Yan et al., 2021).

In vitro applications, selecting the correct PRP dose is key to ensuring its effectiveness (Görgü et al., 2021). Too low a PRP concentration may not produce a significant protective effect, while excessive concen-

tration can cause sperm clumping, increase medium viscosity, or even trigger the activation of inflammatory factors from platelets (Caterino *et al.*, 2023). Several studies have shown that a range of 1–10% (v/v) is often used as a starting point, but its efficacy is greatly influenced by the type of PRP used (e.g., LP-PRP or LR-PRP), its bioactive molecule content, and the length of incubation time (Everts *et al.*, 2020; Şeker *et al.*, 2025). Determining the optimal dose needs to be tailored to the application's purpose, whether to maintain the quality of liquid semen, increase resistance to oxidative stress, or support the refreezing process during cryopreservation (Nagata *et al.*, 2019).

The addition of PRP has a direct effect on motility, viability, morphology, and the level of ROS production in sperm cells (Mirzaei *et al.*, 2022). Growth factors such as IGF-1 and PDGF can increase mitochondrial metabolic activity, thereby supporting increased progressive motility (Aghanoori *et al.*, 2019). Furthermore, the antioxidant components in PRP help reduce ROS production, which, when excessive, can trigger lipid peroxidation, DNA damage, and decreased sperm viability (Bollwein and Bittner, 2018). Several studies also report that PRP can maintain normal sperm morphology by minimizing damage to the acrosome and plasma membrane (Li *et al.*, 2025; Moradian *et al.*, 2025). Overall, PRP functions as a multifunctional bioactive agent that increases sperm resistance to various forms of environmental stress, both during short-term storage and during freezing and thawing (Abdulla *et al.*, 2022).

In Vivo PRP application in males

The *in vivo* application of PRP in bulls is beginning to be considered as a biotherapeutic approach in animal reproduction, although scientific evidence in cattle is still relatively limited (Borş *et al.*, 2022). PRP, which contains various growth factors such as PDGF, TGF- β , IGF-1, and VEGF, has the potential to repair testicular tissue and improve spermatogenesis through regenerative mechanisms and antioxidant effects (Alharbi, 2024). In several species, PRP therapy has been used through direct injection into the testis or epididymis, primarily to address issues such as testicular degeneration, chronic inflammation, and decreased spermatogenic activity (Fazli *et al.*, 2024).

Physiologically, PRP injection into the testicles is thought to increase the proliferation of Sertoli and Leydig cells, two cell types that play a crucial role in sperm formation and testosterone regulation (Demyashkin *et al.*, 2025). The growth factors in PRP can stimulate tissue regeneration, enhance local angiogenesis, and optimize oxygen and nutrient supply to the seminiferous tubules (Patel *et al.*, 2023). This is crucial because the quality of the testicular microenvironment significantly impacts spermatogenesis (Bordbar *et al.*, 2024). Furthermore, PRP's antioxidant properties have the potential to reduce the accumulation of free radicals that typically arise from oxidative stress, a major cause of sperm DNA damage and impaired maturation in the epididymis (Walke *et al.*, 2023).

Several research reports in other species have shown that PRP administration in the epididymal area has the potential to improve epithelial structure, enhance sperm maturation, and maintain cell membrane stability during epididymal storage (Haidar, 2024; Hosseini *et al.*, 2025). These effects are related to the biomolecules contained in PRP, which support epididymal cell regeneration, reduce inflammation, and improve membrane permeability, thus maintaining a favorable environment for sperm maturation (Dehghani *et al.*, 2019).

Comparison of PRP with other compounds or technologies

PRP is now considered a promising candidate for improving bovine semen quality, and its effectiveness needs to be compared with other compounds and technologies long used in semen quality management (Zhu *et al.*, 2024). Conventional antioxidants such as vitamin E, vitamin C, quercetin, and melatonin have been widely used as protective agents during storage and cryopreservation (Cao *et al.*, 2022). The primary mechanisms of action of these compounds include free radical scavenging, membrane lipid stabilization, and protection of sperm DNA from oxidative damage (Wang *et al.*, 2025b). Despite their significant benefits, antioxidant-based approaches are limited by their passive nature and inability to repair existing cellular damage (Muscolo *et al.*, 2024). Table 1 presents a comprehensive comparison of PRP with various bovine semen quality-enhancing agents, including common antioxidants, nanoparticle technology, and stem cell-conditioned media (SCM).

In contrast to traditional antioxidants, PRP contains a diverse array of bioactive molecules that operate through more sophisticated pathways, functioning not only as free radical scavengers but also as stimulators of various biological activities (Everts *et al.*, 2024). Growth factors such as IGF-1 and PDGF contribute to cellular regeneration and enhance mitochondrial efficiency, while VEGF supports angiogenesis in *in vivo* applications (Andrae *et al.*, 2008). These attributes position PRP as a multifunctional agent capable of protecting sperm, repairing structural damage, and boosting metabolic capacity during storage and following thawing (Nabavinia *et al.*, 2023). Even so, the variability in PRP composition among donors poses a significant challenge, unlike synthetic antioxidants which offer more uniform and predictable concentrations (Rossi *et al.*, 2023).

In addition to conventional antioxidants, nanoparticle-based technologies such as zinc oxide nanoparticles (ZnO-NP) and selenium nanoparticles (SeNP) are rapidly emerging as modern approaches to improving semen quality (Abedin *et al.*, 2023). These nanoparticles possess potent antioxidant activity, high bioavailability, and improved stability within semen extenders (Horky *et al.*, 2023). However, potential toxic effects on sperm cell membranes and the possibility of accumulation in reproductive tissues are issues that require caution (Sciorio *et al.*, 2025). In this context, PRP appears to be a safer option because it is derived from natural

Table 1. Comparison of PRP with various compounds and technologies for improving the quality of bovine semen

Category	Agent / Technology	Main mechanism	Advantages	Limitations / Risks
Conventional antioxidants	Vitamin E, Vitamin C, Quercetin, and Melatonin	Scavenging free radicals, stabilizing lipid membranes, and protecting DNA from oxidative stress	Easy to use, inexpensive, stable, and with a lot of scientific evidence	Passive effect, does not repair cellular damage that has already occurred, and effectiveness is limited to conditions of severe oxidative stress
PRP	PRP	Combination of antioxidants, growth factors (IGF-1, PDGF, and VEGF), stimulation of mitochondrial metabolism, and cell regeneration	Multifunctional agent, able to repair cell damage, increase sperm metabolic capacity, and low risk of toxicity because it is biological	Variability in composition between donors, requires a preparation process, and is not yet standardized
Nanotechnology	ZnO-NP and SeNP	High antioxidant activity, increased bioavailability, and stable in extender	The potential for improving the quality of strong and low-dose semen is already effective	Risk of toxicity, potential sperm membrane damage, and accumulation in reproductive tissues
Biotechnology	SCM	Provides paracrine factors, enhances tissue regeneration, reduces ROS, and improves testicular function.	More controlled composition, strong regenerative effect, and potential to improve spermatogenesis	Expensive, requires cell culture facilities, impractical for industrial scale, and complex protocols

biological components and has a low risk of toxicity, although the main challenge remains the consistency and standardization of its components (Vrapcea *et al.*, 2025).

On the other hand, the use of SCM is a modern biotechnological strategy that provides a paracrine factor-rich environment, acting through a mechanism similar to PRP but with a more standardized composition and concentration of growth factors (Everts *et al.*, 2020). SCM has been shown to enhance testicular tissue repair, maintain sperm viability, and reduce oxidative stress levels (Hsiao *et al.*, 2019). However, the development of SCM requires complex cell culture facilities, high production costs, and stringent laboratory procedures, making its application on an industrial scale less efficient than the more easily prepared PRP (Jayaraman *et al.*, 2021). Figure 3 provides a visual comparison of the core mechanisms and constraints of the four primary methods used to enhance bovine semen quality.

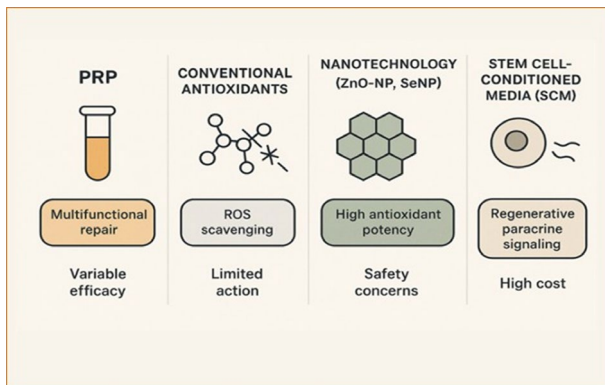


Figure 3. Comparative schematic of four primary methods used to enhance bovine semen quality.

Limitations, challenges, and future research directions

Although PRP has significant potential to improve bovine semen quality, its use remains limited by various methodological constraints and application challenges (Hosseini *et al.*, 2025). Differences in manufacturing methods, variations in biological components, and a lack of standardized studies are key factors hampering consistent results (Alonso-Frías *et al.*, 2025). Therefore, a critical analysis of these limitations, operational challenges in the field, and the establishment of a clearer research direction are crucial steps to maximize the effectiveness of PRP in bovine reproduction.

Limitations of the current study

Although PRP has significant potential to improve bovine semen quality, understanding its use remains limited by several methodological and scientific challenges (Caterino *et al.*, 2023). One major challenge is the variability in PRP preparation techniques (Tey *et al.*, 2022). Differences in procedures, including variations in centrifugation speed, separation duration, anticoagulant type, and activation method—lead to heterogeneity in PRP products (Zhou *et al.*, 2025). This variation affects platelet counts, leukocyte concentrations, and growth factor levels, complicating comparisons between studies and reducing the consistency of results (do Amaral *et al.*, 2016).

Furthermore, research specifically evaluating the effects of PRP on bovine semen, whether in liquid or cryopreserved form, is still very limited (Salama *et al.*, 2024). Most of the available information comes from studies in other species, such as humans, horses, and laboratory animals, making the application of these results to the bovine reproductive system unreliable (Fuertes-Recuero *et al.*, 2025). This limitation creates a knowledge gap regarding the effectiveness of PRP under the unique physiological and biological conditions of bovine bulls, including the sperm response to PRP bioactive molecules and the determination of safe dos-

ages (El-Sherbiny *et al.*, 2022).

Another challenge faced is the differences in PRP concentration and composition between studies (Zhou *et al.*, 2025). Variations in platelet count, plasma proportion, leukocyte presence, and donor blood quality result in inconsistent PRP composition (Tey *et al.*, 2022). These factors influence antioxidant activity, regenerative capacity, and the effectiveness of PRP in repairing sperm membrane integrity and DNA (Moradian *et al.*, 2025). Without standardization, determining the optimal dose and clearly understanding the dose-response relationship for PRP use in bovine semen is difficult (Brito *et al.*, 2025).

Application challenges in industry

The application of PRP in the bovine reproductive industry still faces several significant challenges that must be overcome before this technology can be widely used (Gonçalves *et al.*, 2020). One major obstacle is the need for standardization in PRP production (Zhou *et al.*, 2025). Differences in manufacturing procedures including centrifugation methods, activation techniques, and anticoagulant types result in PRP with highly variable characteristics, both in terms of platelet concentration and bioactive factor content (Tey *et al.*, 2022). Without clear standards, it is difficult for the industry to guarantee the quality, safety, and consistency of PRP used to improve bovine semen (El-Sherbiny *et al.*, 2022). Therefore, developing a uniform and measurable production protocol is crucial to ensuring the successful implementation of PRP on a commercial scale (Sebbagh *et al.*, 2023).

Furthermore, the stability of PRP during storage remains a technical challenge that has not been fully resolved (Qiu *et al.*, 2024). As a biological product, PRP is susceptible to degradation, particularly decreased growth factor activity and changes in plasma protein composition (Xie *et al.*, 2014). Freezing, thawing, and long-term storage can affect the quality of PRP, thus impacting its ability to protect sperm or maintain motility during storage (Castro *et al.*, 2025). The lack of adequate scientific data regarding the optimal storage duration, ideal temperature, and functional stability of PRP is a major obstacle to its development as a ready-to-use product for the artificial insemination industry (Liu *et al.*, 2024).

Furthermore, the application of PRP requires special attention to biosafety and regulatory aspects (Sebbagh *et al.*, 2023). As a blood-derived product, PRP poses a potential risk of microbial contamination, disease transmission, or immunological reactions if not processed hygienically and in accordance with biosafety procedures (Arita *et al.*, 2025). Regulations regarding the use of blood products in livestock are also limited or vary from country to country, necessitating policy harmonization to ensure their safety and appropriateness (Seitz *et al.*, 2008). The industry must ensure that the PRP used meets laboratory quality standards, is free from pathogens, and is produced through a verified system (Patel *et al.*, 2023).

Future research directions

Future research on the use of PRP to improve bovine semen quality should focus on strengthening scientific evidence through a multidisciplinary approach and oriented toward practical application (Santolaria *et al.*, 2023). A key priority is the implementation of large-scale field studies (Yao *et al.*, 2025). Currently, most PRP research is conducted in laboratories with limited sample sizes, which does not reflect the complexity of field variables, such as differences in farm management, environmental variations, and heterogeneity in bull quality (Bollwein and Malama, 2023). Field studies using controlled trial designs with representative cattle populations are essential to assess the effectiveness of PRP in a practical context, including its impact on pregnancy rates, semen efficiency, and overall reproductive performance (Mikkola *et al.*, 2024).

Furthermore, developing a standardized PRP protocol for application to bovine semen is crucial (Caterino *et al.*, 2023). This standardiza-

tion includes PRP preparation methods, optimal platelet ratios, leukocyte content, activation techniques, appropriate dosages, and procedures for mixing PRP with extenders or cryoprotectants (Dhurat and Sukesh, 2014). PRP characterization parameters, including growth factor concentrations, antioxidant activity, and cytokine profiles, need to be established as quality indicators (Wang *et al.*, 2025a). Consistent implementation of protocols will facilitate replication of results, enable comparison between studies, and support more effective PRP adoption in the artificial insemination industry (Ayantoye *et al.*, 2025).

Research is also focused on integrating PRP with modern cryoprotectant technology to improve sperm resistance to freezing and thawing stress (Nabavinia *et al.*, 2023). PRP can be combined with technologies such as nanoparticle-based cryoprotectants, biocompatible polymers, or next-generation membrane-protecting molecules (Reddy *et al.*, 2018). The synergy between PRP and these cryoprotectant agents is expected to provide dual protection, through the bioactive mechanisms of growth factors and cytokines, as well as the physicochemical mechanisms of the cryoprotectant (Zhou *et al.*, 2025). This approach has the potential to reduce membrane damage, improve post-thawing sperm motility, and suppress ROS formation during the preservation process (Yan *et al.*, 2021).

Conclusion

PRP has great potential as a biostimulant to improve bovine semen quality because it contains various growth factors, cytokines, and bioactive molecules that play a role in improving sperm viability, motility, and membrane integrity. Numerous scientific evidence shows that PRP can improve the microenvironment of reproductive cells and provide protection against oxidative stress. The use of PRP also has the potential to support increased reproductive efficiency, particularly in artificial insemination programs that require high-quality semen. However, further research is needed to determine the appropriate dosage, optimal application methods, and evaluate long-term safety before PRP can be widely implemented in bovine reproduction.

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Conflict of interest

The authors declare that there is no conflict of interest.

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