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Platelet-rich plasma therapy in the management and treatment of male infertility—an update of state-of-art

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Abstract: Platelets are small cell fragments in the blood that play a crucial role in blood clotting and wound healing. Through a process called apheresis, where blood is drawn from a donor, separated into its components using a centrifuge, platelet concentrates are obtained. Of those, Platelet-Rich Plasma (PRP) has garnered considerable attention in recent years mainly due to its attractive regenerative and wound healing properties, prompting extensive research in a wide range of applications. Within the realm of human reproduction, PRP can be considered a biofunctional material as it contains cytokines that have demonstrated notable effects on sperm quality and function. This article aims to present a comprehensive literature review on the utilization of PRP for male infertility treatment. A thorough bibliographic search was conducted encompassing articles and reviews published in scientific journals over the last 5 years, focusing on the potential of PRP formulations in addressing male factor infertility. Overall, nine publications were identified, suggesting the PRP application across various domains including sperm cryopreservation, management of oxidative stress, cultivation of spermatogonial stem cells, and in addressing azoospermia (absence of sperm in the ejaculate or semen of a male). Findings indicate that supplementing the cryopreservation media with PRP enhances sperm parameters, while PRP-incubated samples exhibit a heightened antioxidant capacity. Moreover, PRP demonstrates potential in augmenting the proliferation and vitality of spermatogonial stem cells, alongside facilitating sperm recovery in non-obstructive azoospermia cases (where the problem with sperm production lies within the testes themselves and not due to any physical duct obstruction).



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Despite promising outcomes, research with larger sample sizes is warranted to delineate optimal protocols and ascertain the clinical safety, efficacy, and predictability of PRP therapy in male infertility management.

Keywords: platelets; plateletrich plasma; PRP; male infertility; platelet therapy; semenogram; spermatozoa; sperm quality; growth factors; cryopreservation; cytokines; oxidative stress; spermatogonial stem cells; azoospermia; Klinefelter syndrome; vas deferens

1. Introduction

Platelet Concentrates, briefly, are autologous blood extracts obtained through centrifugation of whole blood samples [1–3]. The preparation procedure allows the gathering and concentration of platelets and other therapeutic blood constituents (fibrinogen/fibrin, growth factors, leukocytes and circulating cells), in clinically-usable preparations, biofunctional materials and surgical adjuvants. Despite promising clinical observations, in a wide range of applications and indications, their overall effectiveness remains debated to date [1–5]. This is mainly due to: mixed/variable clinical outcomes, limited high-quality evidence-based literature, and poor characterization of end-products (and preparation protocols) used in studies; and - until recently - lack of proper terminology systems to classify these preparations [1–3]. Indeed, the first “classification” consensus [1] was published in 2009, describing 4 different Platelet Concentrate sub-families, based on variability in biological content (fibrin and cell), properties (gelification) and potential applications: pure platelet-rich plasma (P-PRP), leukocyte and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF) and leukocyte and platelet-rich fibrin (L-PRF) [3–5]. Today, it can be safely stated that, in reproductive medicine, the PRP sub-family is receiving the most attention, mainly due to simplicity, user-friendliness and cost-effectiveness, when compared to the other preparations.

Generally, Platelet Rich Plasma, or PRP, can be simply defined as that fraction of plasma with a higher platelet concentration than that of the blood of a healthy person pre-centrifugation [2–4]. Platelets are small cell fragments in the blood that play a crucial role in blood clotting, wound healing, reparative and regenerative medicine [1–5]. Obtaining PRP is simple and quick since only centrifugation of a blood sample is necessary. Certain components of PRP have been shown to play a crucial role in cellular and tissular repair and regeneration [1–5], with observed positive effects on seminal quality, erectile dysfunction and male infertility [6]. Indeed, PRP is currently used in a wide variety of clinical indications such as extra-/intra-oral surgeries, musculoskeletal injuries, cranio-maxillo-facial reconstruction, and dermatology, and recently its use has been expanded to reproductive medicine, being a treatment already used in certain cases of female infertility. Henceforth, the use of PRP therapy has been accompanied with an increasing interest and demand in understanding its efficacy, mechanism(s) of action and potential clinical application, for an improved quality of life of millions of patients worldwide [7–9]. Therefore, the purpose of this literature review is to comprehensively filter and analyze the current state-of-art and research efforts related to the application of PRP as a therapeutic option in male infertility indications. Key aspects will be addressed, including the description of PRP, its preparation

and storage, its current clinical use, and the potential mechanism(s) through which PRP could improve the semenogram parametric of patients, *i.e.* male fertility, based on clinical data from published studies. Limitations and prospects for this emerging area are also discussed.

2. Methods

Studies included in this mini-review article were obtained from the PubMed[®]/MEDLINE[®] database, using the following search keywords: “Platelet-rich plasma (PRP)”, “PRP and male infertility”, “PRP and male fertility”, “PRP and infertility”, “PRP and male fertility”, “PRP and spermatozoa”, “PRP and erectile dysfunction”, “PRP and sperm cryopreservation”, “PRP and oxidative stress”, “PRP and semenogram”, “PRP and spermatogonial cell culture”, “PRP and spermatogonia”, “PRP and azoospermia”, “PRP and obstructive azoospermia”, “PRP and non-obstructive azoospermia”, “PRP and idiopathic infertility”, “PRP and testicular tissue injury”. The search period for English—as well as Spanish-language publications between the years 2018 and 2023 resulted in less than ten articles, of which four employed an animal model (*in vivo/pre-clinical*) and four were identified as clinical trials in humans. Hits were initially screened by title and abstract, followed by tabulation and a thorough examination of selected and included articles; where the *in vitro*—only studies were excluded. Given the lack of standardization in PRP preparation protocols, special attention to variations in qualitative and quantitative observations and reported semen parametric was conducted, to deliver, to the best of capabilities, a comprehensive and updated review of the state-of-art.

3. Results

3.1. PRP definition, description, and history in the recent literature

The use of PRP can be dated back to the 1970s, when it was used in thrombocytopenic patients. Interestingly, PRP was initially developed in the field of maxillo-facial surgery and oral (dental) implantology, gaining more and more attention for its ability to enhance localized tissue regeneration and promote wound healing [3–5]. At that time, it was defined as plasma with a platelet concentration greater than that found in peripheral blood [6]. Indeed, the concept of PRP revolves around harnessing the regenerative potential of the platelets found in blood. Platelets contain various growth factors and cytokines that play crucial roles in tissue repair and regeneration, hence, via isolating and concentrating platelets from own blood of an individual patient, PRP can be obtained/created and then applied to targeted areas of injury or dysfunction [1–5]. Over the years, PRP therapy has expanded beyond oral and dental surgery to various medical disciplines, including orthopaedics, dermatology, sports medicine, and aesthetics. Its versatility and potential benefits have led to extensive research and clinical applications in treating conditions such as tendon injuries, osteoarthritis, chronic wounds, and hair loss, amongst others, such as the topic or focus of this review, *i.e.* male fertility. Today, PRP is perhaps better described as the fraction of plasma with a higher platelet concentration when compared to that of the blood of a healthy subject prior to centrifugation. Technically, a platelet concentration five times greater than the initial

preparation, at least around 1×10^6 cell/ μ l, is considered or classified as a PRP formulation [10]. Nonetheless, it is worth mentioning that multiple classifications have been proposed in the literature. Herein, categorizing PRP is based on different parameters, such as: purity of the obtained PRP sample, its platelet and/or leukocyte content, concentration and/or quantity, exogenous activation, and the presence of erythrocytes. The most recent recommendation from the Scientific Committee on Standardization of the International Society on Thrombosis and Hemostasis or ISTH, aimed at standardization, primarily for regenerative medicine applications, comprises using products containing platelets or platelet-rich concentrates [11].

3.2. PRP preparation, activation, handling, storage, formulation, and application protocol

While reported protocols may/do vary depending on the intended application and the preferences of healthcare providers, a typical process regularly includes the following steps: **(1) Blood Collection:** A small volume of whole blood is drawn from the patient, usually from a vein in the arm, using a sterile technique; **(2) Centrifugation:** The collected blood is then centrifuged to separate its components based on their density. This process typically involves spinning the blood at high speeds to separate red blood cells, platelets, and plasma; In terms of centrifugation protocols for use in male infertility applications, it is noteworthy herein that those are not completely defined, to date, and to the best of knowledge. This is mainly due to inconclusiveness of the reported results in available studies and literature. Nevertheless, a recent 2021 article from the IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce), shedding some light regarding centrifugation protocols, obtained promising results through the following protocol: To obtain PRP, set speed at 380 g for 15 minutes to separate the blood into 3 layers followed by a second centrifugation at a speed of 1300g for 8 minutes. Data demonstrated that semen incubated with PRP for 1 hour had better mobility and quality, when compared to fresh semen [12]. **(3) Platelet Concentration:** After centrifugation, the platelet-rich fraction is isolated from the rest of the blood components. This fraction contains a higher concentration of platelets compared to whole blood; **(4) Activation:** Optional where in some protocols, the isolated PRP may be activated using various agents such as calcium chloride, thrombin, or collagen, which can trigger the release of growth factors from the platelets; **(5) Application:** Once prepared, the activated or non-activated biofunctional PRP material can be applied directly to the target defect or area of treatment. This may involve injection, topical application, or incorporation into other medical procedures, depending on the specific medical condition being addressed; and **(6) Storage:** PRP and platelet concentrates, in general, have a limited shelf life, usually around 5–7 days, and are typically stored at room temperature. Thus, it must be handled and transfused with care to prevent bacterial contamination and ensure efficacy as in therapeutics.

Herein, in reproductive medicine applications, the preparation of PRP often begins with a blood extraction using an anti-coagulant in the extraction system, normally a sodium citrate solution, avoiding platelet activation before application. From here, there are two processing options, depending on whether one or two consecutive centrifugations are performed [13]. The 1st centrifugation cycle is aimed to separate the collected blood into 3 distinct levels or

portions, as is illustrated in Figure 1; Bottom level contains red blood cells (RBCs) and leukocytes just above it, Middle or intermediate level corresponds to PRP, and Top or upper level composed of platelet-poor plasma or PPP. Middle and Top portions are to be preserved for their plasma and leukocyte content, discarding the Lower portion. If a 2nd centrifugation of the Middle or intermediate layer is to be performed in the adopted protocol, then the PPP supernatant and the platelet pellet with some RBCs will result, which then will be re-suspended to obtain the PRP [7]. Here, an agonist can be utilized for platelet activation [14]. It is critical to note that despite the various existing methods and protocols for PRP preparation, the basic requirements recommended by the literature “consensus” comprise a platelet concentration 4 to 6 times higher than the initial sample, with leukocytes absent or below 1000 cell/ml and erythrocytes at levels equal to or less than 100 cell/ml [10,13]. Finally, it is noteworthy that a platelet concentration 6x higher than initial preparation should not be exceeded; as it could, contrary to belief, exert an inhibitory effect on the target therapy [13].

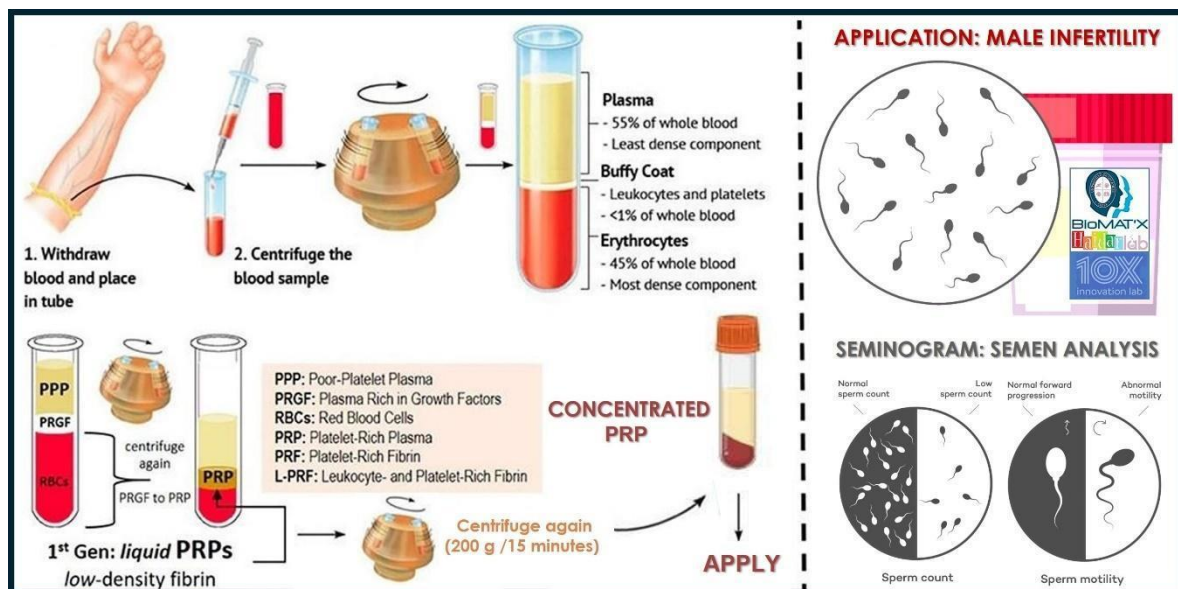


Figure 1. Scheme for obtaining PRP preparations or formulations from withdrawn blood via performing one to two centrifuges. Briefly, the first phase comprises separating the collected blood into three levels: the lower one with red blood cells and leukocytes in the upper part; the intermediate corresponds to PRP and the upper would be composed of platelet-poor plasma or PPP. The lower level is then discarded, and the two upper portions preserved. If a second centrifugation of the intermediate level is performed, the PPP supernatant and the platelet pellet with some red blood cells will be obtained, which will be then re-suspended to obtain the highly-concentrated PRP. Please note that an agonist can be used to activate platelets. Illustration created using PowerPoint and BioRender.

Technical Note: Formulation-wise, PRP can vary in terms of platelet concentration, white blood cell content, and the presence or absence of activating agents. These variations can influence the biological activity and therapeutic effects of PRP. Additionally, factors such as the

type of centrifuge used, centrifugation parameters (e.g., speed and duration), and the anti-coagulants employed during blood collection can also impact the final PRP bio-formulation.

3.3. Clinical application of PRP: from oro-maxillo-facial surgery to reproductive medicine

As aforementioned, PRP is currently mainly used in oral and maxillofacial surgery, orthopaedics, plastic reconstructive surgery and in other musculo-skeletal injuries suffered by professional athletes, *i.e.* sports medicine. PRP is also routinely used in dermatology and aesthetics, for the improvement of scars and recalcitrant wounds [2–6]. Recently, PRP have demonstrated promising effectiveness in cardiac, spinal pain management applications [11].

While scientific evidence, including long-term data on safety and efficacy in clinical applications, supports these areas, PRP, as a biofunctional material, remains *experimental* within the realm of reproductive medicine, in general, and for male patients, in specific. Indeed, the predominant focus of R&D&I (research, development, and innovation) has centered on female infertility, particularly stemming from low ovarian reserve, recurrent implantation failures, or thin endometrium [7–9]. In these clinical scenarios, PRP has demonstrated the ability to elevate the anti-Müllerian hormone or AMH (also known as Müllerian-inhibiting hormone or MIH) levels, reduce the Follicle-Stimulating Hormone (FSH) levels, augment endometrial thickness, enhance clinical pregnancy rates, and exhibit a tendency toward increasing live birth rates [8]. Generally, studies tend to indicate that PRP holds potential and promise in ameliorating reproductive outcomes through endometrial regeneration, bolstering receptivity, enhancing folliculogenesis (process by which ovarian follicles develop and mature within the ovaries), and in addressing select cases of intra-uterine adhesions. However, to transition PRP from an experimental to a validated treatment, randomized controlled trials with larger cohorts and long-term data are imperative [7,9,14]. In the context of male infertility, although clinical efficacy of PRP and long-term outcomes are still being studied, some research suggests that PRP injections into the male reproductive tract, such as the testes or the epididymis, may have the potential to improve sperm quality. As will be discussed later in this article, PRP contains growth factors and cytokines that could stimulate spermatogenesis (sperm production) and enhance sperm motility and morphology. Other studies opted to employ or utilize PRP therapy as a complementary or adjunctive treatment alongside existing infertility interventions, such as, ART or assisted reproductive technologies including *in vitro* fertilization or IVF and/or ICSI or intra-cytoplasmic sperm injection. However, similar to other studies and applications, its role in improving outcomes in conjunction with these treatments remains to be fully elucidated, before it can be routinely recommended as a standard treatment option. Also, clinical studies evaluating the effects of PRP on sperm parameters, reproductive outcomes, and potential adverse effects are ongoing.

3.4. Experimental nature of PRP: from cytokines to reducing ROS and DNA fragmentation

Several studies investigating the application of PRP for male infertility have highlighted its positive impact on growth factors and other platelet components, which in turn have demonstrated favorable effects on sperm parameters. Notably, platelet-derived factors have

been associated with enhancements in sperm function and quality. Additionally, research indicates that within the testes, PRP can mitigate ischemia, expand the width of seminiferous tubules, maintain the integrity of the germinal epithelium, and modulate the function of Sertoli and Leydig cells [15,16]. For instance, fibroblast growth factor (FGF-2), when incubated with sperm, was reported to enhance their progressive motility. Additionally, when introduced in conjunction with epidermal growth factor (EGF), it stimulated the Sertoli cells to release the androgen-binding protein, thus facilitating spermatogenesis [16, 17]. In another notable example, insulin-like growth factor 1 (IGF-1) boosted sperm viability and mobility. Herein, its incorporation into the freezing media improved membrane and mitochondrial integrity, as well as DNA stability [11]. Furthermore, IGF-1 contributed to the proliferation of the Sertoli cells, augmenting their numbers, renewal, and longevity [18]. Moreover, the vascular endothelial growth factor (VEGF), another cytokine, increased motility and reduced oxidative stress during spermatogenesis, *in vitro*, yet did not enhance sperm survival [18,19]. Serotonin, on the other hand, found in PRP, accelerated the curvilinear speed of sperm [16]. An effect suggested to stem from its ability to elevate glucose consumption in skeletal muscle, thereby inducing a similar effect on sperm and enhancing a rapid head movement [16]. Furthermore, the platelet enzymes integrated into the cryopreservation media have been reported to enhance the post-thaw sperm parameters. For example, SOD or superoxide dismutase reduced the reactive oxygen species (ROS) levels, a damage process triggered by cold. Such a reduction in peroxidation helps prevent DNA fragmentation in the cryopreserved sperm and thereby results in decreased levels of non-viable sperm [19]. Please remember that these observations stem from various independent and distinctive studies, underscoring the presence of growth factors and cytokines in PRP that have the potential to stimulate spermatogenesis (sperm production) and improve sperm motility and morphology. Consequently, speculation that injecting PRP into testicular tissue could promote spermatogenesis and aid in restoring sperm production in individuals experiencing impaired spermatogenesis within the testes, is growing. Similarly, this proposed notion supports that PRP injections into penile tissue may promote tissue regeneration, enhance blood flow, and potentially ameliorate erectile dysfunction (while not directly related to male infertility, PRP therapy has also been investigated as a treatment for erectile dysfunction, which can contribute to difficulties in achieving pregnancy). Yet, despite the hope and/or hype, it is crucial to reiterate herein that PRP therapy for male infertility remains experimental, requiring further research to ascertain safety, effectiveness, and optimal treatment regimens.

3.5. PRP therapy in the management and treatment of male infertility: from lab to clinic

3.5.1 Improvement of sperm freezing and cryo-preservation

Enhancing sperm freezing or cryopreservation with PRP is an area of ongoing R&D&I in the field of male infertility, amongst others (see Table 1). Briefly, sperm cryopreservation intends to allow the seminal sample from the patient to be preserved for future use in artificial insemination or *in vitro* fertilization treatments as well as for preserving fertility in cancer patients who require to undergo radio- or chemo-therapy and/or any immuno-suppressive

strategies, or prior to a vasectomy [9,19]. PRP, through its cellular- and tissue-regenerative properties may potentially help the overall reproductive system, via repairing any damage to the testicular or epididymal tissue caused by the freezing process, thereby enhancing overall sperm quality and fertility potential. So, to summarize, PRP can help improve sperm cryopreservation via:

(a) protecting against oxidative stress; (b) enhancing sperm survival; and (c) promoting sperm function(s). Certainly, throughout the freezing and thawing cycles/stages, significant changes take place within the cellular milieu or environment, inducing osmotic stress on the sperm cells, thereby compromising their structural integrity and membrane flexibility/fluidity. Moreover, glycolysis is also impeded and inhibited, leading to diminished or reduced cellular ATP levels and consequently a reduction in sperm vitality and motility [20,21]. Addressing these challenges, the scientific community has long sought improving the technical methods to mitigate the adverse effects of sperm cryopreservation. Numerous and perhaps most studies have explored the use of various supplements and cryoprotectants, with promising outcomes. For example, in a recent 2022 study [20], semen samples were obtained from buffaloes, and then investigated comparatively various concentrations of autologous PRP (0%, 2%, 5%, and 10%), before and after freezing. Upon thawing, the semen parameters were compared to assess the impact of PRP. Results indicated that samples treated with 5% PRP exhibited superior sperm quality post-thaw when compared to semen samples treated with the other PRP concentrations. Specifically, these semen samples demonstrated increased progressive motility, enhanced membrane vitality and integrity, and reduced acrosomal defects as well as reduction in total abnormalities. Moreover, an elevated superoxide dismutase (SOD) enzyme activity, enhanced antioxidant capacity, and decreased lipid peroxidation were reported. Furthermore, oocytes inseminated with sperm samples cryopreserved with 5% PRP exhibited higher fertilization rates, improved embryonic division, increased blastocyst formation, and lower polyspermy rates, compared to controls. El-Sherbiny and colleagues concluded that the utilization of 5% PRP during semen cryopreservation significantly enhances both sperm quality and fertilizing potential [20]. In another 2023 study also employing buffaloes, Almadaly and group [22] investigated a wider range of PRP concentrations (0%, 5%, 10%, 15%, 20%, and 25%) for distinct (fertile *versus* infertile) semen sample cryopreservation. At the study's onset, animals were categorized as either fertile or sub-fertile. Notably, a concentration of 15% PRP demonstrated significant improvements in buffalo semen quality, including speed, antioxidant, and fertilizing potential/capacity. Furthermore, the researchers assessed the levels of IGF-1 in both semen groups. As aforementioned, IGF-1, a key component of PRP, has been shown to enhance sperm and acrosomal membrane integrity, DNA stability, and sperm motility. Interestingly, the sub-fertile group exhibited lower IGF-1 levels when compared to the fertile group, suggesting a potential correlation between IGF-1 plasma concentration and male fertility [22]. Furthermore, R&D&I into the use of PRP for semen cryopreservation has extended to intra-testicular injection, in rabbits, where Abdulla and group [23] aimed to assess mobility parameters and the expression of genes associated with rabbit sperm synthesis and cellular stress regulation, since specific genes typically experience reduced expression during the

cryopreservation process. In this study [24], rabbits treated with PRP exhibited enhanced progressive motility, total motility, hyperactivation, and sperm viability, when compared to the control group. Additionally, PRP-treated rabbits demonstrated lower levels of DNA fragmentation and improved gene expression, reaching levels comparable to those prior to freezing. Such interesting findings further underscores the protective effect of autologous PRP in preserving animal sperm samples during the freezing /cryopreservation process [23]. As depicted in Table 1, the potential of PRP is multi-variate.

Table 1. Potential of biofunctional PRP Application in Male Reproductive Medicine.

Use of PRP in Male Infertility Indications	Brief Description of Main Observation
Treatment of Secretory Azoospermia	Intratesticular injection of PRP may improve sperm cell recovery and implantation rates.
Enhancement of Sperm Quality	PRP supplementation in cell culture may enhance sperm motility, viability, and proliferation.
Improvement of Seminal Cryopreservation	PRP may mitigate oxidative stress and improve sperm quality in cryopreserved samples.
Potential Use in Prepubertal Cancer Patients	PRP could offer fertility preservation options
Treatment of Non-obstructive Azoospermia	PRP therapy may address sperm motility issues

Currently, there are no long-term studies or clinical trials specifically supporting the use of PRP for sperm cryopreservation in humans. However, R&D&I in the field of sperm cryopreservation is ongoing, focusing on various methods and improvements to enhance the quality and viability of preserved sperm. Sperm cryopreservation has been a significant area of interest since the 1950s, primarily involving techniques such as slow freezing and vitrification. The primary challenges associated with cryopreservation include cryo-damage from ice crystal formation and oxidative stress, which can impair sperm motility, viability, and DNA integrity [25,26]. Herein, it is worth mentioning that while PRP is being explored for its regenerative properties in clinical indications, its application in sperm cryopreservation remains largely *experimental*, to date. Current laboratory-scale and bench-top advancements focus on optimizing cryoprotectant constituent(s) and formulations and incorporating anti-oxidants to help mitigate/avoid any cryo-damage and improve post-thaw sperm quality [27].

It is noteworthy herein that studies involving humans are limited in number and typically encompass small participant cohorts. In 2023, Nabavinia and colleagues [18], obtained 20 semen samples from healthy normozoospermic men aged 20 to 30 years. To clarify, “normozoospermic” refers to subjects with normal sperm parameters within the semen. This includes factors such as sperm count, sperm motility (the ability of sperm to move), sperm morphology (the shape and structure of sperm), and other characteristics evaluated during a semenogram (semen analysis). Samples diluted with a PRP concentration of $1 \times 10^5 \mu\text{l}$ indicated significantly improved sperm motility and viability, along with a reduction in the percentage of immotile and non-progressive sperm, when compared to control. Interestingly, a decrease or reduction in PRP concentration was associated with an increase in abnormal sperm and a decrease in protamines (which are small, positively charged proteins found in

the nucleus of sperm cells). Additionally, a rise in sperm DNA fragmentation was observed with decreasing platelet content [18]. In 2021, Yan and group [9] utilized 12 normozoospermic samples, dividing them into 4 aliquots to which autologous PRP concentrations of 0%, 2%, 5%, and 10% were added. Following sample thawing, various parameters including vitality, motility, membrane integrity, DNA fragmentation, mitochondrial membrane potential, and ROS levels were assessed. The results revealed that the inclusion of 5% PRP during incubation significantly elevated values in the semen samples when compared to the control group. Notably, a significant enhancement/improvement in membrane integrity is suggested to be attributed to the protein component of the PRP, which possesses inherent protective properties against the crystallization and heating encountered during cryopreservation. While positive, the detected improvements in DNA fragmentation, ROS levels (Reactive Oxygen Species are chemically reactive molecules containing oxygen, such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals), and mitochondrial membrane were not statistically significant in this study [9]. It is perhaps worth mentioning herein that in the context of sperm, ROS levels refer to the concentration of these reactive oxygen species within the obtained/collected seminal fluid sample. Different concentrations of PRP have been shown to have varying effects on sperm quality. Generally, moderate concentrations such as 5% and 15% PRP have demonstrated significant improvements in sperm motility, vitality, and overall quality during cryopreservation and oxidative stress conditions. On the other hand, lower concentrations, including 2% PRP, have also been effective in reducing oxidative stress-related damage in sperm cells. [28,29]. Nevertheless, it is advisable to continue studying the effects that exist in relation to the different concentrations in order to continue identifying the benefits in each of them. While low levels of ROS are necessary for normal physiological processes, excessive ROS production can lead to oxidative stress, which can damage sperm cells by causing lipid peroxidation, protein oxidation, and DNA damage. On the other hand, high ROS levels in semen samples have been associated with male infertility, as they can impair sperm function, decrease sperm motility, and affect sperm DNA integrity. Therefore, measuring ROS levels in semen samples can provide valuable insights into oxidative stress and its potential impact on male fertility.

3.5.2 ROS, oxidative stress and DNA damage in human spermatozoa and male infertility

Briefly, oxidative stress, characterized by an imbalance between cellular antioxidant capacity and the presence of Reactive Oxygen Species or ROS, stands as a significant contributor to male infertility. This imbalance can lead to detrimental effects such as DNA damage and alterations in sperm membrane structure. These changes are also implicated in reduced fertilization rates, impaired embryonic development, and even miscarriage, according to recent literature [23,24]. Excessive ROS production triggers the activation of caspases and apoptosis, along with lipid peroxidation, which compromises sperm membrane fluidity, subsequently diminishing sperm motility and its ability to fuse with the oocyte (the immature form of an egg/germ cell and is found within the female ovaries) [24]. Thus far, various antioxidants have been proposed to counteract and/or alleviate the damage caused by

oxidative stress in male infertility, including PRP treatment, given its minimally-invasive accessibility, and abundant content of bio-active and -functional substances capable of mitigating such deleterious effects. Indeed, in a recent study [23], researchers conducted an *in vitro* experiment in which 30 semen samples were incubated with varying doses of H₂O₂ to determine the concentration and duration required to induce oxidative stress in sperm samples. Subsequently, they treated both the H₂O₂-exposed and unexposed samples with increasing concentrations of PRP (0%, 2%, 5%, and 10%). Notably, seminal parameters exhibited greater improvement when treated with 2% PRP in both groups, showing increased total mobility and progressive mobility, as well as reductions in ROS-positive cells, vacuolization, DNA fragmentation, and apoptotic cell count. These findings suggest that autologous PRP enhances sperm quality under conditions of oxidative stress induced by H₂O₂. Such reported effects of PRP on seminal quality align with findings reported in other assays (see Table 2), where the sperm parametric improved following treatment with PRP.

Table 2. PRP use and resultant semen parametric: outline of selected studies and trials.

Authors & Pub year	Model	PRP conc. ^a	PRP preparation protocol	Progressive mobility (%)	Viability	DNA fragment. (%) ^b	ROS+ve cells ^c	Membrane Integrity (%)	REF
ANIMAL SEMEN SAMPLES – pre-clinical ASSAYS									
El-Sherbiny <i>et al.</i> 2022	8 fertile buffaloes	5%	Double centrifugation and platelet activation 10 ⁹ cell/ml PRP	48.28%	64.72%	N/A	N/A	61.36%	[19]
Almadaly <i>et al.</i> 2023	11 buffaloes; 8 fertile, 3 sub-fertile buffaloes	15%	Double centrifugation	Fertile mobility: 55.34%; Sub-fertile mobility: 55.12%	Fertile viability: 79.20%; Sub-fertile viability: 75.83%	N/A	N/A	Fertile: 47.83%; Sub-fertile: 47.16%	[21]
Abdulla <i>et al.</i> 2022	20 rabbits PRP inj. (intra-testicular)	N/A	(Biozek Medical®, Laan van de Ram, Bulgaria) 4000 × 10 ⁹ platelets/μl	34.60%	59.40%	14.90%	N/A	N/A	[22]
HUMAN SEMEN SAMPLES – in vitro ASSAYS									
Nabavinia <i>et al.</i> 2023	20 normozoospermic males	1 × 10 ⁵ platelets/μl	(Rooyagen, Tehran, Iran) 1 × 10 ⁵ , 0.5 × 10 ⁵ and 0.25 × 10 ⁵ platelets/μl	~40%	~65%	~25%	N/A	N/A	[18]
Yan <i>et al.</i> 2021	12 normozoospermic male	5%	Double centrifugation	30.30%	65.50%	18.80%	14.10%	52.40%	[9]
Bader <i>et al.</i> 2020	30 healthy males; cultured with H ₂ O ₂	2%	Double centrifugation 10 ⁶ platelets/μl	~45%	~65%	20%	N/A	30%	[23]

^a Best reported result/finding; ^b DNA fragmentation percentage; ^c ROS, reactive oxygen species.
N/A: Not Applicable and/or Not Reported in Study.

3.5.3 Spermatogonia, the proliferation of spermatogonial stem cells and spermatogenesis

Briefly, spermatogonia are a type of germ cell found in the testes of males, located along the inner wall of the testicular seminiferous tubules [15,18]. They are the stem cells of spermatogenesis (spermatogonial stem cells or SSC), the process by which sperm cells are produced. Spermatogonia undergo mitotic divisions to maintain the population of stem cells and to produce spermatocytes, which then undergo further divisions to eventually develop into mature sperm cells. They can divide either to self-renew and maintain the pool of spermatogonia or to differentiate into primary spermatocytes, which marks the beginning of the process of sperm cell development [15,18]. Henceforth, spermatogonia are essential for the continuous production of sperm throughout the reproductive lifespan of a male. So, those males diagnosed with cancer, for example, and who often face the necessity of undergoing harsh cytotoxic therapies, especially if early in life, can significantly have their present and future fertility impacted by adversely affecting the SSC cargo. In reproductive medicine and R&D&I laboratories, there is a clear interest in exploring or developing novel methods and techniques to enhance SSC proliferation and differentiation, *in vitro*, before clinical transplantation, in an attempt to improved nutrient transport and overcome the limiting and finite quantity of these vital cells [18]. Therefore, and continuing along this trajectory, the utilization of PRP as a supplement in both 2D and 3D cell culture of SSC has been examined. Figure 2 below aims to summarize the main advantages of 2D *versus* 3D cell culture systems.

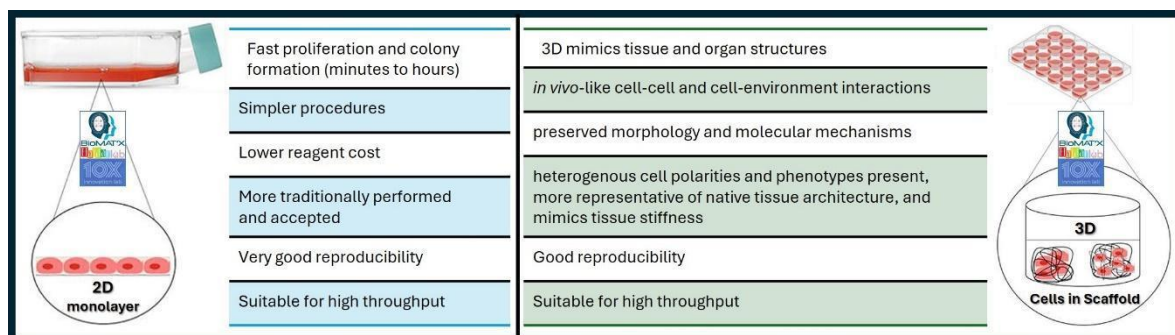


Figure 2. Advantages of 2D *versus* 3D cell culture systems. Briefly, 3D cell culture provides an improved platform for studying physiological aspects such as the cell cycle, cell proliferation, apoptosis, cell adhesion and cell motility. In 2D cell cultures, there is no cellular microenvironment, and cells are forced into planar shape, henceforth, does not mimic native structures. Also, complex cell-cell and cell-environment interactions are lacking in 2D cell culture systems, when compared to the superior yet more costly and slower (culture formation due to physical restraints of the matrix requires hours to days) 3D culture systems. In gene and protein expression studies, 3D cell culture is a much safer and representative option for mimicking the *in vivo* and human expression of genes and proteins. Figure, deemed worthy to emphasize 2D *vs.* 3D, created using PowerPoint and BioRender.

Khadivi *et al.* [18], isolated samples from four testis donors and then cultured with varying concentrations of PRP (1%, 2.5%, 5%, and 10%). Notably, enhanced proliferation and viability of SSCs were observed with 5% PRP. Once the optimal PRP concentration was determined, the samples were then divided into three groups: 2D culture without PRP, 2D culture with PRP, and 3D culture with PRP. Significantly higher levels of proliferation and viability were noted in the 3D group treated with 5% PRP, leading to increased cell survival over the course of the culture assay. Moreover, a significant upregulated expression of the genes responsible for proliferation was observed, suggesting that PRP supplementation promotes an organizational structure that enhances cell migration, resembling the natural microenvironment of the SSC niche. Nonetheless, the researchers reported herein that while the diameter of colonies was deemed larger in the 2D culture with PRP, this may be attributed to the significantly greater number of the colonies observed in the 3D culture with PRP [18].

In 2019, Dehghani and group [15], induced sterility in 32 mice via administering busulfan to testes. PRP was then injected into select groups, leading to observed enhancements, when compared to the control group, including elevated testosterone levels, proliferation of SSC, elongation of sperm tails, and increased motility. Herein, the augmented number of SSC corresponded to an overall increase in cells across the entire lineage, encompassing spermatogonia A and B, spermatocytes, and spermatids [15]. The study concluded that PRP injection holds promise in treating infertility resulting from severe apoptosis of sperm cells, a phenomenon occurring naturally with age and exacerbated by factors like oxidative stress or exposure to toxic substances (as in cancer therapy). Yet, with a call for further research work to substantiate the findings and elucidate the interrelationships among these processes.

Technical Note: The effectiveness of PRP in the treatment of male infertility can vary significantly between different species due to differences in the concentration of platelets (*i.e.* species that physiologically have a greater amount of platelets tend to respond better), growth factors (difference in the expression of genes that encode growth factors is regulated at the transcriptional and translational level, and these processes differ between species), proteins and cytokines within the PRP, as well as in the biological characteristics (semen quality, motility and morphology differ between species and can vary sensitivity to PRP) of the male reproductive system (the vascularization and cellular composition of testicular and epididymal tissue vary between species, which could lead to differences in how PRP interacts with tissues) [28–31]. Further, variations in semen quality, reproductive tract structure, and immune response between species may influence how PRP interacts with target tissues and cells. Therefore, it is important to know that it is necessary to perform more species-specific studies to determine the optimal concentrations and appropriate composition of PRP, as well as to better understand the interaction of its components with reproductive tissues [32,33].

3.5.4 Impaired sperm production and secretory azoospermia: from ART to surgery to PRP

The treatment of secretory azoospermia, a condition characterized by the absence of sperm in ejaculate due to impaired sperm production, has been also explored in the literature.

Typically, stimulating sperm production involves addressing the underlying cause if identifiable, and some of the potential approaches include:

(a) medical management of underlying conditions, such as infections, autoimmune disorders or genetic conditions that may be contributing to secretory azoospermia can sometimes aid in restoring sperm production; (b) hormonal therapy, recommended if the azoospermia is due to hormonal imbalances, such as low levels of testosterone or elevated levels of follicle-stimulating hormone or FSH; (c) surgical intervention, when deemed necessary to correct anatomical abnormalities that hinder sperm production or transport, such as varicocele repair for normal blood flow in the scrotum; (d) ART, or Assisted Reproductive Techniques including *in vitro* fertilization or IVF with intra-cytoplasmic sperm injection or ICSI may be considered if sperm cannot be retrieved from the ejaculate (other sperm retrieval techniques are available, such as testicular sperm extraction (TESE) or microdissection TESE (micro-TESE or mTESE) can be used to retrieve sperm directly from the testes for use in IVF-ICSI); and (e) healthy life-style modifications, including maintaining a balanced diet, regular exercise, avoiding excessive alcohol consumption, and avoiding exposure to environmental toxins, may help improve sperm production and overall fertility. Herein, PRP has been recently proposed for use in cases of secretory azoospermia. In 2022, Gudelci *et al.* [34] explored the intra-testicular injection of PRP and its effects on 135 men diagnosed with non-obstructive azoospermia who had undergone at least one unsuccessful micro-TESE procedure. Briefly, the azoospermic patients were divided into two groups: Group 1, comprising individuals with one prior micro-TESE failure, and Group 2, consisting of those with two or more prior failures. In Group 1, sperm cells were identified in 27.5% of cases, with an implantation/live birth rate of 7.5%. However, in Group 2, success rates were lower, with sperm cells identified in 8.3% of cases and an implantation/live birth rate of 3.6%. Findings suggest that this treatment approach may benefit men with non-obstructive azoospermia and a history of unsuccessful micro-TESE procedures by improving sperm cell recovery and implantation/live birth rates [34]. It is noteworthy that treatment plans for secretory azoospermia should be individualized based on the specific underlying causes and the preferences of the individual or couple. Also, please remember that despite the published evidence supporting the efficacy of PRP in male infertility indications and its growing popularity, the scientific, technical, and clinical evidence across different medical specialties remains variable, with some studies showing positive outcomes while others demonstrate inconclusive results. Thus, limitations and shortcomings exist. Findings, interpretations, and conclusions are to be taken in with great caution, and unquestionably, randomized controlled clinical trials are necessary. Consequently, ongoing efforts are to hopefully focus on refining and validating the bio-functional prep methods, standardizing R_x protocols, and elucidating its mechanism(s) of action; to optimize the therapeutic utility(ies) of PRP, and other PCs [4].

Technical Note: The clinical contraindications for using PRP (Platelet-Rich Plasma) in male fertility therapies are not well-documented. Most studies report no local adverse events such as erythema or blockage following PRP injections. However, patients with blood disorders affecting platelet function may not fully benefit from PRP treatment due to its reliance on a sufficient number of functional platelets. Additionally, administering PRP to

patients with systemic infections or infected areas could exacerbate these conditions. Current research shows promising results, indicating that PRP may improve sperm quality and testicular function, presenting new treatment possibilities. Despite these advances, more research is needed, particularly long-term studies and larger clinical trials, to fully understand the potential and limitations of PRP in male fertility therapies. Even so, the outlook is positive, and continued R&D&I could establish PRP as a significant tool in combating male infertility.

4. Conclusion

The present concise review shows, despite scarcity of literature, an accruing interest in investigating as well as incorporating the use of PRP as a biofunctional material and therapeutic modality in the management and treatment of infertility-related indications and conditions in male patients. Studies on the use of PRP during cryopreservation or exposure to oxidative stress showed an improvement in sperm quality, including viability, mobility, membrane, and DNA integrity. Furthermore, in animal models, an increase in fertilization rates has been observed. When incubating spermatogonial stem cells with PRP, studies suggested a benefit in cancer patients who resort to the culture of these cells to preserve their fertility before undergoing cytotoxic therapy as in cases of non-obstructive azoospermia. Research has also shown that PRP may enhance sperm quality, increase sperm motility and viability, and improve outcomes in ART. Moreover, PRP has demonstrated potential in treating conditions such as secretory azoospermia, where conventional treatments have yielded limited success. Generally, these findings are consistent, to an extent, with previous works showing that the growth factors present intrinsically in PRP promote cell proliferation and differentiation, and hence are bio-actively and-functionally involved in the repair and regeneration processes. Nonetheless, despite the encouraging results, there is a plethora of limitations in these studies. To list a few, sample size is often relatively small and the technical protocols for obtaining PRP are diverse and not standardized, and in many cases are not described, which hinders the proper comparison of results. Carrying out more *in vitro*, *in vivo*, and *ex vivo* assays and conducting human trials are recommended and awaited, for more robust evidence, to guarantee the comparability and reproducibility of the findings, and to facilitate decision-making (cracking hope *versus* hype) by the attending healthcare provider in reproductive medicine, for male patients and couples facing fertility challenges.

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Conflicts of interests

Author discloses no potential conflict(s) of interest of any shape or form.

Abbreviations

AMH, anti-Müllerian hormone; EGF, epidermal growth factor; FGF-2, fibroblast growth factor 2; FSH, follicle-stimulating hormone; IGF-1, insulin-like growth factor; PC: platelet concentrate; PPP, platelet-poor plasma; PRP, platelet-rich plasma; ROS, reactive oxygen species; SSC, spermatogonial stem cells; mTESE, microdissection testicular sperm extraction; 2D, two-dimensional; 3D, three-dimensional.

Ethical statement

This work is exempt from any Ethics Committee and/or Institutional Review Board approval as it is deemed neither necessary nor required.

References

- [1] Ehrenfest DMD, Rasmusson L, Albrektsson T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol.* 2009, 27(3):158–167.
- [2] Bielecki T, Ehrenfest DMD, Everts PA, Wiczowski A. The role of leukocytes from L-PRP/L-PRF in wound healing and immune defense: New perspectives. *Curr. Pharm. Biotechnol.* 2012, 13(7):1153–1162.
- [3] Zumarán CC, Parra MV, Olate SA, Fernández EG, Muñoz FT, *et al.* The 3 R's for platelet-rich fibrin: a “super” tri-dimensional biomaterial for contemporary naturally-guided oro-maxillo-facial soft and hard tissue repair, reconstruction and regeneration. *Materials.* 2018, 11(8):1293.
- [4] Haidar ZS. L-PRF: A “Super” Biomaterial for Naturally Guided Hard/Soft Tissue Bioengineering and Regeneration of Oro-Dental, Periodontal and Jaw Defects in *Bone Grafting-Recent Advances with Special References to Cranio-Maxillofacial Surgery.* London: IntechOpen, 2018.
- [5] Muñoz F, Jiménez C, Espinoza D, Vervelle A, Beugnet J, *et al.* Use of leukocyte and platelet-rich fibrin (L-PRF) in periodontally accelerated osteogenic orthodontics (PAOO): Clinical effects on edema and pain. *J. Clin. Exp. Dent.* 2016, 8(2):e119–e124.
- [6] Alves R, Grimalt R. A review of platelet-rich plasma: history, biology, mechanism of action, and classification. *Skin Appendage Disord.* 2018, 4(1):18–24.

- [7] Sharara FI, Lelea LL, Rahman S, Klebanoff JS, Moawad GN. A narrative review of platelet-rich plasma (PRP) in reproductive medicine. *J. Assist. Reprod. Genet.* 2021, 38(5):1003–1012.
- [8] Epifanova MV, Gvasalia BR, Durashov MA, Artemenko SA. Platelet-rich plasma therapy for male sexual dysfunction: myth or reality?. *Sex. Med. Rev.* 2020, 8(1):106–113.
- [9] Yan B, Zhang Y, Tian S, Hu R, Wu B. Effect of autologous platelet-rich plasma on human sperm quality during cryopreservation. *Cryobiology.* 2021, 98(105):12–16.
- [10] Martínez-Martínez A, Ruiz-Santiago F, García-Espinosa J. Platelet-rich plasma: myth or reality? *Radiology.* 2018, 60(6):465–475.
- [11] Everts P, Onishi K, Jayaram P, Lana JF, Mautner K. Platelet-rich plasma: new performance understandings and therapeutic considerations in 2020. *Int. J. Mol. Sci.* 2020, 21(20):1–36.
- [12] Angellee J, Ginting CN, Chiuman L, Halim B. Role of platelet-rich plasma to sperm quality in male partners undergoing infertility treatment. In *Proceedings of 2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce)*, Medan, Indonesia, 14–16 July, 2021, pp. 1–5.
- [13] Dhurat R, Sukesh M. Principles and methods of preparation of platelet-rich plasma: a review and author's perspective. *J. Cutan. Aesthet. Surg.* 2014, 7(4):189.
- [14] Magalon J, Chateau AL, Bertrand B, Louis ML, Silvestre A, *et al.* DEPA classification: a proposal for standardizing PRP use and a retrospective application of available devices. *BMJ Open.* 2016, 2(1):1–5.
- [15] Dehghani F, Sotoude N, Bordbar H, Panjeshahin MR, Karbalay-Doust S. The use of platelet-rich plasma (PRP) to improve structural impairment of rat testis induced by busulfan. *Platelets.* 2019, 30(4):513–520.
- [16] Hernández-Corredor L, León-Restrepo S, Bustamante-Cano J, Báez-Sandoval G, Jaramillo X. Effect of the incorporation of plasma rich of platelets on the spermatozoa physiology of ram cum. *J. Dairy Vet. Anim. Res.* 2020, 9(9):34–38.
- [17] Susilowati S, Triana IN, Malik, A. The effects of insulin-like growth factor I (IGF-I) complex from seminal plasma on capacitance, membrane integrity and DNA fragmentation in goat spermatozoa. *Asia Pac. J. Manag.* 2015, 4(3):208–211.
- [18] Khadivi F, Koruji M, Akbari M, Jabari A, Talebi A, *et al.* Application of platelet-rich plasma (PRP) improves self-renewal of human spermatogonial stem cells in two-dimensional and threedimensional culture systems. *Acta Histochem.* 2020, 122(8):151627.

- [19] Nabavinia MS, Yari A, Ghasemi-Esmailabad S, Gholoobi A, Gholizadeh L, *et al.* Improvement of human sperm properties with platelet-rich plasma as a cryoprotectant supplement. *Cell Tissue Bank.* 2023, 24(2):307–315.
- [20] El-Sherbiny HR, Abdelnaby EA, Samir H, Fathi M. Addition of autologous platelet rich plasma to semen extender enhances cryotolerance and fertilization capacity of buffalo bull spermatozoa. *Theriogenology.* 2022,194:104–109.
- [21] Gómez-Torres MJ, Medrano L, Romero A, Fernández-Colom PJ, Aizpurúa J. Effectiveness of human spermatozoa biomarkers as indicators of structural damage during cryopreservation. *Cryobiology.* 2017, 78:90–94.
- [22] Almadaly EA, Ibrahim IM, Salama MS, Ashour MA, Sahwan FM, *et al.* Effect of platelet-rich plasma (PRP) on post-thaw quality, kinematics and in vivo fertility of fertile and subfertile buffalo (*Bubalus bubalis*) spermatozoa. *Vet. Res. Commun.* 2023, 47(1):61–72.
- [23] Abdulla AK, Rebai T, Al-Delemi DHJ. Protective effects of autologous platelet-rich plasma (PRP) on the outcome of cryopreservation in rabbit sperm. *Cell. Mol. Biol.* 2022, 68(4):113–121.
- [24] Bader R, Ibrahim JN, Moussa M, Mourad A, Azoury J, *et al.* In vitro effect of autologous platelet-rich plasma on H₂O₂-induced oxidative stress in human spermatozoa. *Andrology.* 2020, 8(1):191–200.
- [25] Ozimic S, Ban-Frangez H, Stimpfel M. Sperm cryopreservation today: approaches, efficiency, and pitfalls. *Curr. Issues Mol. Biol.* 2023, 45(6):4716–4734.
- [26] Antonouli S, Nisio VD, Messini C, Daponte A, Rajender S, *et al.* A comprehensive review and update on human fertility cryopreservation methods and tools. *Front. Vet. Sci.* 2023, 10:1151254.
- [27] Bojic S, Murray A, Bentley BL, Spindler R, Pawlik P, *et al.* Winter is coming: the future of cryopreservation. *BMC Biol.* 2021, 19:1–20.
- [25] Cho CL, Agarwal A. Role of sperm DNA fragmentation in male factor infertility: a systematic review. *Arab J Urol.* 2017, 16(1):21–34.
- [26] Wegener J, May T, Kamp G, Bienefeld K. A successful new approach to honeybee semen cryopreservation. *Cryobiology.* 2014, 69(2):236–242.
- [27] Samy A, El-Adl M, Rezk S, Marghani B, Eldomany W, *et al.* The potential protective and therapeutic effects of platelet-rich plasma on ischemia/reperfusion injury following experimental torsion/detorsion of testis in the Albino rat model. *Life Sci.* 2020, 256:117982.

- [28] El-Sherbiny HR, Abdelnaby EA, Samir H, Fathi M. Addition of autologous platelet rich plasma to semen extender enhances cryotolerance and fertilizing capacity of buffalo bull spermatozoa. *Theriogenology*. 2022, 194:104–109.
- [29] Almadaly EA, Ibrahim IM, Salama MS, Ashour MA, Sahwan FM, *et al.* Effect of platelet-rich plasma (PRP) on post-thaw quality, kinematics and in vivo fertility of fertile and subfertile buffalo (*Bubalus bubalis*) spermatozoa. *Vet Res Commun*. 2023, 47(1):61–72.
- [30] Bos-Mikich A, Oliveira RD, Frantz N. Platelet-rich plasma therapy and reproductive medicine. *J. Assist. Reprod. Genet*. 2018, 35(5):753–756.
- [31] Ulhe SM, Choudhary N, Shrivastava J, Dutta S, Dakre SM, *et al.* Overcoming male factor infertility: a journey through assisted reproductive technology with platelet-rich plasma therapy. *Cureus*. 2024, 16(3):e55378.
- [32] Sekerci CA, Tanidir Y, Sener TE, Sener G, Cevik O, *et al.* Effects of platelet-rich plasma against experimental ischemia/reperfusion injury in rat testis. *J. Pediatr. Urol*. 2017, 13(3):317.e1–317.e9
- [33] Akhigbe RE, Odetayo AF, Akhigbe TM, Hamed MA, Ashonibare PJ. Pathophysiology and management of testicular ischemia/reperfusion injury: lessons from animal models. *Heliyon*. 2024, 10(9):e27760.
- [34] Gudelci T, Cakiroglu Y, Yuceturk A, Yazicioglu C, Karaosmanoglu O, *et al.* Men with non-obstructive azoospermia and history of failed (mTESE) microdissection testicular sperm extraction: outcomes after intratesticular injection of platelet rich plasma (PRP). *Fertil Steril*. 2022, 118(4):e289.