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Efficacy of platelet-rich plasma on blastocyst formation in ICSI cycles involving low-quality sperm: A randomised study

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ABSTRACT

Objective: To evaluate the efficacy of platelet-rich plasma (PRP) incubation on embryo developmental outcomes in intracytoplasmic sperm injection (ICSI) cycles involving low-quality sperm.

Methods: Sixty-six couples undergoing ICSI were enrolled in a prospective randomised study conducted at Halim Fertility Center, Indonesia between January and August 2023. Male with severe oligoasthenoteratozoospermia were randomised into two groups. Following density gradient centrifugation, samples in the PRP group were incubated with autologous PRP for one hour prior to ICSI, whereas the non-PRP group underwent standard processing without PRP exposure. Baseline characteristics and assisted reproductive technology outcomes between two groups were compared. The primary outcome was the blastocyst formation rate, while secondary outcomes included fertilisation rate, cleavage rate, and blastocyst quality.

Results: A total of 66 couples were included, with 33 couples in each group. Participants' demographics and semen parameters did not differ significantly between the two groups. The PRP group demonstrated a higher fertilisation rate than the non-PRP group (84.1% vs. 78.2%), but the difference was not statistically significant ($P=0.38$). The PRP group showed significantly higher cleavage rates (98.9% vs. 95.0%; $P=0.03$) and blastocyst formation rates (56.7% vs. 46.6%; $P=0.006$) compared to the non-PRP group. Additionally, the PRP group exhibited a higher proportion of good-quality blastocysts (44.8% vs. 41.2%; $P=0.01$) and a lower proportion of poor-quality blastocysts (22.1% vs. 27.8%; $P=0.03$).

Conclusions: PRP incubation prior to ICSI in severe oligoasthenoteratozoospermia cases improves cleavage rates, blastocyst formation, and embryo quality. PRP may be a promising adjunct to optimise ICSI outcomes in male factor infertility.

Study registration: This study was registered at ClinicalTrials.gov with the registration number NCT07579858.

KEY WORDS: Platelet-rich plasma; Severe oligoasthenoteratozoospermia; Sperm quality; Blastocyst formation; Embryo development

Key Points

Question: Can the incubation of platelet-rich plasma (PRP) improve embryological outcomes in intracytoplasmic sperm injection (ICSI) cycles using sperm from males with severe oligoasthenoteratozoospermia?

Findings: In this randomised prospective study involving 66 couples, sperm samples that were incubated with PRP prior to ICSI exhibited significantly higher cleavage rates (98.9% vs. 95.0%), blastocyst formation rates (56.7% vs. 46.6%), and a greater proportion of high-quality blastocysts (44.8% vs. 41.2%) than controls, but there was no significant difference in fertilisation rates.

Meaning: PRP incubation enhances both blastocyst development and the quality of embryos in cases of sperm from males with severe oligoasthenoteratozoospermia. This suggests that PRP could serve as a valuable supplementary approach to optimise outcomes in ICSI procedures.

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1. Introduction

Platelet-rich plasma (PRP) is a concentrated suspension of platelets in a small amount of plasma derived from autologous blood. It is essential for tissue repair due to its abundant growth factors that enhance cell proliferation, facilitate tissue regeneration, and expedite the healing process. Since its introduction in clinical applications during the 1980s and 1990s, primarily in cardiac, dental, and maxillofacial surgery, PRP therapy has gained extensive application across various fields of regenerative medicine[1].

Sperm quality is a crucial determinant of *in vitro* fertilisation (IVF) outcomes, influencing every stage from fertilisation to subsequent embryo development. Extensive evidence demonstrates that poor semen parameters adversely affect embryogenesis, leading to decreased rates of fertilisation, cleavage, and blastocyst formation[2,3]. Among these parameters, sperm progressive motility and morphology are particularly important. Abnormalities in these parameters are strongly associated with diminished blastocyst development and suboptimal embryo quality[4]. Notably, the impact of sperm quality on embryo development is evident even before embryonic genome activation, suggesting the essential contribution of paternal factors to the early stages of successful embryogenesis[2].

Intracytoplasmic sperm injection (ICSI) represents the standard intervention for male factor infertility, particularly in cases involving diminished sperm concentration, motility, or morphology. Yet, despite significant advances in assisted reproductive technology (ART), suboptimal sperm quality remains a persistent impediment to clinical success. Deficiencies in semen parameters are frequently associated with fertilisation failure, impaired embryonic development, and reduced blastocyst formation. Clinical evidence suggests that severely low sperm concentration correlates with reduced fertilisation and blastocyst formation rates in ICSI cycles, while diminished motility further attenuates fertilisation efficacy. Additionally, patients exhibiting poor sperm quality regularly demonstrate markedly reduced blastocyst formation rates compared to those with normal semen parameters[5].

Oxidative stress is widely acknowledged as a primary driver of sperm dysfunction, as increased levels of reactive oxygen species (ROS) lead to lipid peroxidation and impair fertilisation potential and embryo quality[6]. Elevated ROS levels are directly correlated with increased deoxyribonucleic acid (DNA) fragmentation and reduced motility, both of which severely attenuate ICSI success rates[7]. Moreover, sperm DNA integrity, typically assessed through assays such as the Comet assay, has emerged as a predictive indicator of subsequent embryo quality and overall IVF success[8]. These findings collectively necessitate the exploration of novel and effective strategies to improve sperm functionality before ICSI. PRP supplementation, with its regenerative and anti-oxidative properties, represents a promising therapeutic strategy that may improve sperm parameters and clinical results, particularly in cases of severe

oligoasthenoteratozoospermia (SOAT).

The regenerative capabilities of PRP are well-documented across numerous tissues; nevertheless, its application in andrology remains limited and underexplored. Initial research indicates that the key growth factors contained within PRP, namely platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF- β), and insulin-like growth factor-1 (IGF-1), may improve sperm concentration and motility whilst simultaneously mitigating oxidative stress[9]. However, robust direct evidence supporting its efficacy in augmenting sperm function before ICSI remains scarce, thereby necessitating rigorous additional research to confirm its therapeutic potential.

Our previous work indicated that incubating semen samples with PRP for one hour led to substantial enhancements across several key sperm parameters, including total motility, progressive motility, morphology, and the DNA fragmentation index (DFI)[10]. Specifically, PRP incubation resulted in a 59.5% increase in sperm motility, a 1.9% improvement in sperm morphology, and a 32.5% decrease in the DFI. In light of these encouraging preliminary findings, we undertook a subsequent study to precisely assess the effect of PRP on downstream embryo development, specifically focusing on blastocyst formation and resultant embryo quality.

The present study, therefore, aims to investigate the subsequent efficacy of PRP incubation on fertilisation, cleavage, and blastocyst formation rates in ICSI cycles involving men with SOAT. We hypothesise that enriching the sperm's microenvironment with PRP before ICSI will augment sperm quality and subsequently lead to enhanced embryological outcomes. By specifically assessing blastocyst development and embryo quality between sperm samples treated with PRP and those untreated, this research seeks to ascertain whether PRP represents a potential adjunct for improving ICSI success rates in challenging cases of male factor infertility. We propose that PRP incubation may augment sperm motility and functionality, resulting in enhanced blastocyst formation and overall embryo quality in patients undergoing ICSI.

2. Methods

2.1. Study design

A prospective study of couples undergoing IVF-ICSI cycles was conducted at Halim Fertility Center, Stella Maris Women's and Children's Hospital, Medan, Indonesia. Couples were recruited using consecutive sampling between January 2023 and August 2023. Couples who had previously undergone more than one IVF-ICSI cycle were excluded from participation.

2.2. Inclusion and exclusion criteria

Male partners were screened to determine eligibility based on the

following inclusion criteria: (1) sperm concentration of less than five million/mL; (2) total sperm motility < 42%; (3) sperm normal morphology of less than four percent; and (4) provided informed consent to participate in the study. Exclusion criteria for male partners were applied to those presenting with: (1) leukospermia; (2) a history of HIV, hepatitis, or other reproductive tract infections; (3) a history of utilising drugs or therapies within the past three months, such as antibiotics, radiotherapy, chemotherapy, psychiatric medications, or anabolic steroids; (4) a history of fever within the past three months; (5) a history of testicular carcinoma; (6) using a second ejaculate sample for ICSI; (7) using a cryopreserved semen sample for ICSI; (8) a history of retrograde ejaculation; or (9) a history of coagulopathy.

Female partners were also evaluated for eligibility and were excluded if they satisfied any of the following conditions: (1) aged 40 years or older; (2) had two out of the three conditions mentioned by the Rotterdam criteria for diagnosing polycystic ovarian syndrome (PCOS); or (3) presented low ovarian reserve, indicated by an antral follicle count (AFC) of fewer than five follicles or an anti-Müllerian hormone (AMH) level of < 0.5–1.1 ng/mL.

2.3. Groupings

Upon fulfilling the inclusion and exclusion criteria, eligible male participants were randomly assigned to two groups. Group A consisted of sperm samples incubated with PRP one hour before ICSI, and Group B consisted of sperm samples that received no PRP treatment.

Confounding variables were mitigated through randomisation and the stringent imposition of the inclusion and exclusion criteria outlined above. Randomisation was executed by the embryologist responsible for sperm preparation before ICSI, using registration number parity recorded in the electronic medical record system: odd numbers were allocated to the PRP group and even numbers to the non-PRP group. To ensure objectivity, the embryologists performing the ICSI procedure and assessing the subsequent outcomes remained blinded to group allocation.

2.4. Measuring primary and secondary outcomes

The primary outcome assessed in this study was the blastocyst formation rate. The secondary outcomes included the fertilisation rate, the cleavage rate, and the proportion of good, fair, and poor-quality blastocysts.

2.5. Sperm and PRP preparation

Semen samples were obtained from male partners using sterile, wide-mouthed plastic containers following masturbation in a designated room within the fertility facility to mitigate external influences on semen parameters. A routine semen analysis was

conducted in accordance with the World Health Organisation (WHO) 2010 criteria to evaluate semen volume, concentration, motility, and morphology[11]. Samples diagnosed with SOAT were then processed using the density gradient centrifugation (DGC) technique, incorporating 90% and 45% gradient layers. The samples underwent centrifugation at 300 *g* for 5 min, a refinement employed to minimise potential DNA fragmentation[12]. Following centrifugation, the supernatant was meticulously removed, and the remaining pellet was retained for subsequent use.

To prepare the PRP, 10 mL of autologous peripheral blood was obtained from each male partner and transferred into a sterile 14 mL round-bottom tube filled with 0.5 mL of anticoagulant. The blood underwent a two-step centrifugation process. The initial centrifugation was performed at 380 *g* for 15 min to segregate the blood into plasma, buffy coat, and red blood cell layers. The uppermost plasma layer was retained and subsequently centrifuged at 1300 *g* for 8 min. Following this, two-thirds of the upper part of the resultant plasma was discarded, and the platelet-rich lower part was homogenised to procure the final PRP product. For sperm incubation, a total of 30 µL of the prepared PRP was subsequently added to 0.3 mL of the processed sperm sample (one-part PRP to ten-part prepared semen). The PRP group samples were then incubated at room temperature for one hour before ICSI.

2.6. Ovum-pick up (OPU) and ICSI

Female partners enrolled in the IVF-ICSI cycles underwent controlled ovarian stimulation (COS) using the gonadotropin-releasing hormone (GnRH) antagonist protocol, followed by final oocyte maturation. The antagonist protocol was initiated on day two of the menstrual cycle, with the gonadotropin dosage ranging from 150 IU to 300 IU daily. A dose of 0.25 mg of the GnRH antagonist was administered daily once at least one follicle had reached a mean diameter of 14 mm, and continued until a minimum of three follicles attained a mean diameter of 17 mm. Final oocyte maturation was triggered by administering 250 mcg of human chorionic gonadotropin (hCG). Oocyte retrieval was subsequently performed 35 to 36 hours after the hCG administration.

The oocytes collected were incubated for 2 hours at 37 °C (6% CO₂ and 5% O₂) before enzymatic denudation with hyaluronidase to facilitate the removal of cumulus cells. All mature oocytes at the metaphase II (MII) stage, excluding those exhibiting giant morphology, were then inseminated by ICSI using the previously prepared sperm samples. This insemination was carried out 38 to 42 hours after the hCG administration.

2.7. Embryo culture and incubation

Fertilisation was evaluated (17±1) hours after ICSI by the identification of two distinct pronuclei (2PN) and the presence of the second polar body (PB). Embryo quality assessments were

performed on day 3 [(68±1) hours after ICSI] and day 5 [(116±2) hours after ICSI]. On day 5, embryo quality was morphologically assessed using the Gardner grading scale. This evaluation focused on the developmental stage (extent of blastocyst expansion), alongside the distinct appearance and cellular integrity of the inner cell mass (ICM) and the trophectoderm (TE) cells. Embryos were then categorised based on the morphological appearance of the ICM and TE cells into three quality bands: good quality (AA), fair quality (AB; BA; BB), or poor quality (AC; CA; BC; CB; CC). To ensure objectivity, the fertilisation check, cleavage assessments, and blastocyst grading were all performed by a single, experienced embryologist who remained blinded to group allocation throughout the entire study period.

2.8. Statistical analysis

Statistical analysis was conducted utilising Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was performed to assess the normality of data distribution. Given the nature of the data, the Mann-Whitney *U* test, *t*-test, and *Chi*-squared test were subsequently applied to evaluate the demographics (age, BMI, pre-OPU oestradiol, pre-OPU progesterone) of the participating couples, the quantity of oocytes retrieved, the number of mature (MII) oocytes, the primary outcome (blastocyst formation rate), and the secondary outcomes (fertilisation rate, cleavage rate, blastocyst quality) of ICSI between PRP and

non-PRP groups. Continuous data are presented as mean±standard deviation (mean±SD) or median (IQR), while categorical data are expressed as percentages (%). The level of statistical significance was established at five per cent ($P<0.05$).

2.9. Ethics statement

This study received ethical approval from the Institutional Ethics Committee of Stella Maris Women's and Children's Hospital (Ethical Number 608.1/Dir/RSIA.SM/XII/2022), granted on 19th December 2022. The study was registered at ClinicalTrials.gov with the registration number NCT07579858.

3. Results

3.1. Participants' demographics

Sixty-six couples were enrolled in this research and randomly allocated into two different groups. Group A (PRP group) consisted of 33 couples where the sperm samples were incubated with PRP one hour before ICSI, while Group B (non-PRP group) consisted of the remaining 33 couples where the sperm samples received no PRP treatment. Figure 1 shows the study flowchart.

The demographics of the couples included paternal age [(36.1±6.1) years *vs.* (37.1±1.2) years], paternal body mass index (BMI)

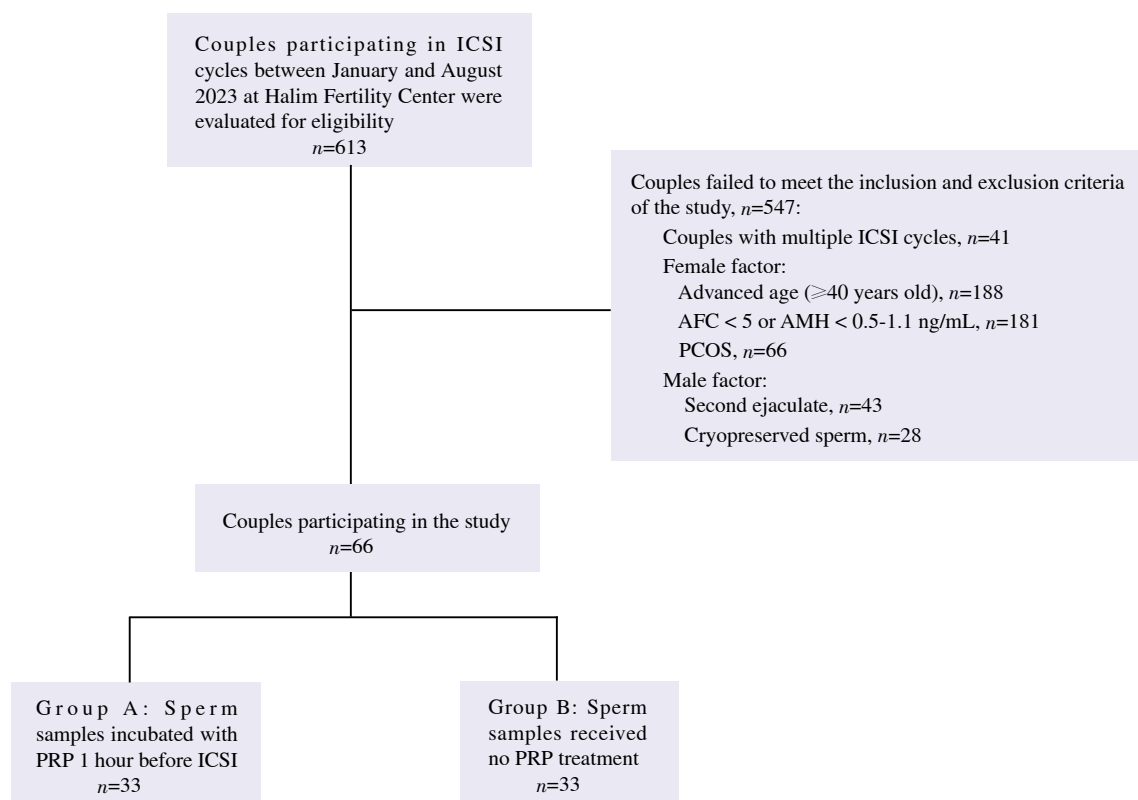


Figure 1. The study flowchart. ICSI: intracytoplasmic sperm injection; AFC: antral follicle count; AMH: anti-Müllerian hormone; PCOS: polycystic ovarian syndrome; PRP: platelet-rich plasma.

Table 1. Participants' demographics.

Variables	PRP group (n=33)	Non-PRP group (n=33)	P-value
Male			
Paternal age, years	36.1±6.1	37.1±1.2	0.35
Paternal BMI, kg/m ²	29.17±2.33	29.80±2.08	0.22
Female			
Maternal age, years	32.6±3.8	33.8±4.9	0.29
Maternal BMI, kg/m ²	25.25±5.87	24.66±5.69	0.68
Pre-OPU oestradiol level, pg/mL	2644 (1818-3470)	2187 (1291-3083)	0.15
Pre-OPU progesterone level, ng/mL	1.00 (0.35-1.65)	0.70 (0.44-0.96)	0.10

Data are expressed as mean±SD or median (IQR); BMI: body mass index; PRP: platelet-rich plasma; OPU: ovum pick-up.

Table 2. Distribution of sperm and oocyte samples.

Variables	PRP group (n=33)	Non-PRP group (n=33)	P-value
Sperm concentration, millions/mL	3.44±0.58	3.58±0.56	0.40
Sperm total motility, %	31.43±2.40	30.85±1.70	0.17
Oocytes retrieved, <i>n</i>	598.0±3.5	486.0±2.6	0.47
Metaphase II oocytes, <i>n</i>	429.0±3.2	357.0±1.7	0.62

Data are expressed as mean±SD. PRP: platelet-rich plasma.

Table 3. ICSI outcomes between two groups.

Variables	PRP group (n=33)	Non-PRP group (n=33)	P-value
Fertilisation rate	84.1	78.2	0.38
Cleavage rate	98.9	95.0	0.03
Blastocyst rate	56.7	46.6	0.006
Good-quality blastocyst	44.8	41.2	0.01
Fair-quality blastocyst	27.3	36.8	0.72
Poor-quality blastocyst	22.1	27.8	0.03

Data are expressed as %. ICSI: intracytoplasmic sperm injection; PRP: platelet-rich plasma.

[(29.17±2.33) kg/m² *vs.* (29.80±2.08) kg/m²], maternal age [(32.6±3.8) years *vs.* (33.8±4.9) years], maternal BMI [(25.25±5.87) kg/m² *vs.* (24.66±5.69) kg/m²], pre-OPU oestradiol [2644 (1818-3470) pg/mL *vs.* 2187 (1291-3083) pg/mL], and progesterone levels [1.00 (0.35-1.65) ng/mL *vs.* 0.70 (0.44-0.96) ng/mL]. No statistically significant differences were observed between the PRP and non-PRP groups in baseline demographic parameters (all *P*>0.05) (Table 1).

3.2. Distribution of sperm and oocyte samples

The sperm concentration [(3.44±0.58) million/mL *vs.* (3.58±0.56) million/mL] and total motility [(31.43±2.40)% *vs.* (30.85±1.70)%] between the PRP and non-PRP groups did not differ significantly (*P*>0.05), nor did the number of oocytes retrieved (598.0±3.5 *vs.* 486.0±2.6; *P*=0.47) and metaphase II oocytes (429.0±3.2 *vs.* 357.0±1.7; *P*=0.62), as shown in Table 2.

3.3. Comparison of ICSI outcomes between groups

The fertilisation, cleavage, and blastocyst formation rates were

calculated for each participant and subsequently averaged across all samples. Table 3 indicates that the fertilisation rate (84.1% *vs.* 78.2%) between the PRP and non-PRP groups did not differ significantly (*P*=0.38). However, a significantly higher percentage of cleavage rate (98.9% *vs.* 95.0%; *P*=0.03) and blastocyst rate (56.7% *vs.* 46.6%; *P*=0.006) could be seen in the PRP group, compared to the non-PRP group. Additionally, the PRP group demonstrated a significantly higher percentage of good-quality blastocysts (44.8% *vs.* 41.2%; *P*=0.01) and a lower percentage of poor-quality blastocysts (22.1% *vs.* 27.8%; *P*=0.03) compared to the non-PRP group.

4. Discussion

The quality of spermatozoa is fundamental in influencing embryo development and the overall efficacy of ART, particularly in IVF-ICSI cycles. Male factor infertility accounts for approximately 50% of all infertility cases, irrespective of accompanying female-related factors, thereby highlighting the necessity of optimising sperm quality to improve clinical outcomes. Extensive evidence demonstrates that poor semen parameters, specifically low sperm concentration, compromised motility, and aberrant morphology, correlate with attenuated fertilisation rates, impaired cleavage, and poor embryo quality[2,5]. Beyond the initial fertilisation event, spermatozoa contribute significantly to embryo morphology and blastomere division. A robust positive association exists among the quality of oocytes, sperm, and embryos[13]. Sperm contributions to embryonic development encompass genetic and epigenetic alterations, mitochondrial and centriole transmission, oocyte activation, and early embryo viability[14,15]. Conversely, genetic anomalies, such as sperm aneuploidy, increased DNA fragmentation, and epigenetic dysregulation, might adversely affect subsequent embryo development[16].

Sperm motility is recognised as a crucial factor influencing both fertilisation and embryo development[5,17]. Low-quality semen is associated with reduced blastocyst formation rates and lower probabilities of producing viable embryos. Overall sperm quality serves as a robust predictor of successful blastocyst development and is highly correlated with the probability of obtaining at least one viable blastocyst in an ART cycle[17]. Significantly, even when fertilisation occurs, suboptimal semen characteristics have been associated with impaired embryonic development[18].

In the present study, PRP administration resulted in a significant improvement in embryological outcomes compared with the non-treated group. Although the fertilisation rate in the PRP group exhibited an upward trend, this difference was not statistically significant. This observation suggests that the treatment may not alter the fundamental process of oocyte activation. The primary determinant of oocyte activation and consequent fertilisation is the delivery of the sperm-specific factor phospholipase C zeta. Upon gamete fusion, phospholipase C zeta hydrolyses phosphatidylinositol

4,5-bisphosphate (PIP₂), producing inositol 1,4,5-triphosphate (IP₃) to trigger the intracellular calcium oscillations required to break oocyte dormancy. Crucially, the expression levels of phospholipase C zeta are established during spermiogenesis within the testes[19,20]. As mature spermatozoa are transcriptionally and translationally quiescent, they lack the biosynthetic capacity to generate *de novo* proteins post-ejaculation. Consequently, incubation with PRP, despite being rich in growth factors, cannot increase the quantitative content of phospholipase C zeta within the sperm and appears to be strictly cytoprotective rather than activating.

Our results align with prior studies, showing the significance of sperm quality in blastocyst development. The ICSI outcomes in the PRP-treated sperm group exhibited markedly superior cleavage and blastocyst formation rates, alongside a higher proportion of high-quality blastocysts. These results underscore the efficacy of PRP in improving post-fertilisation embryogenic competence. Embryo quality is a paramount determinant of IVF success[21]. While suboptimal embryo quality does not seem to elevate the risk of negative obstetric or perinatal outcomes in singleton pregnancies, it does considerably reduce the likelihood of attaining clinical pregnancy and live birth[22]. Morphological assessment is fundamental to evaluating embryo quality, primarily concentrating on factors such as cell count and fragmentation[21].

A 2016 study indicated enhanced implantation rates with blastocyst-stage embryo transfer in comparison to earlier-stage transfers[23]. Furthermore, the quantity of retrieved oocytes and the morphology of day three embryos have been demonstrated to predict blastocyst development, since increased oocyte yields provide superior embryo selection and increase the implantation chances[24]. Consequently, our research primarily focused on evaluating the blastocyst formation rate, a key indicator of embryonic developmental ability. Various factors affect an embryo's likelihood of developing to the blastocyst stage, such as advanced maternal age[25], paternal age[26], endometriosis[27], reduced ovarian reserve[28], and suboptimal sperm quality[5]. Although infertility is complex, sperm quality is a key determinant in successful embryo development.

The findings of this study highlight the therapeutic importance of PRP as a method to improve sperm functionality and subsequent embryo quality. The comparative analysis of PRP and non-PRP groups highlights the capacity of PRP to boost both blastocyst development and embryo quality, as demonstrated by markedly superior outcomes in the PRP-treated sperm group. Sperm motility, governed by hormonal and paracrine regulations, is a vital factor in successful fertilisation. Growth factors have demonstrated beneficial effects in facilitating spermatogenesis[29–31]. PRP, loaded with growth factors, has been utilised in different aspects of male infertility, including mitigation of oxidative stress, stimulation of spermatogonial stem cell proliferation, and restoration of spermatogenesis in cases of non-obstructive azoospermia[32]. The administration of IGF-1, a growth factor contained in PRP, for two months in males with SOAT has been documented to enhance semen

volume, sperm concentration, and progressive motility[33]. Similarly, testicular injections of PRP have also been demonstrated to improve sperm concentration, motility, and DNA integrity in males diagnosed with SOAT[9]. In contrast to these invasive techniques, our study assessed the *in vitro* incubation of sperm with PRP as a non-invasive alternative and illustrated its potential to enhance embryological outcomes.

Our previous investigation demonstrated that PRP incubation markedly enhanced sperm characteristics, including concentration, motility, morphology, and DNA integrity[10]. Subsequent research has validated these findings: A 15-minute incubation with two percent PRP resulted in enhanced progressive and total motility[34]; a one-hour incubation with PRP conducted with glass wool filtration improved progressive motility and morphology while decreasing immotile sperm[35]; and another study reported a 55% increase in progressive motility after 45 minutes of PRP exposure[36]. A meta-analysis of these investigations determined that PRP incubation markedly improves sperm progressive motility and may also enhance concentration and morphology, although the latter was not consistently statistically significant[37].

In the present study, the addition of 30 µL of PRP to 0.3 mL of prepared sperm results in a 10% (v/v) final proportion. This ratio was chosen as it provides a concentrated dose of platelet-derived bioactive factors while ensuring adequate exposure to the growth factors derived from PRP without excessively diluting the sperm suspension or altering its biochemical properties. Practically, 30 µL is readily handled with standard laboratory pipettes, facilitating reproducible addition across samples. Higher proportions may lead to excessive plasma content, potentially changing the viscosity of the prepared sperm, which impairs sperm motility. In contrast, lower proportions may not provide sufficient concentrations of growth factors and antioxidants to exert measurable biological effects.

This study possesses certain limitations, notably the comparatively small and uniform sample size. An expanded and more heterogeneous patient population would enhance the generalisability and reliability of our findings. A further limitation is that the precise blood cell composition, platelet concentration, and the specific type and levels of growth factors present in the PRP preparation were not analytically quantified. Furthermore, subsequent research should focus on establishing the standardised therapeutic range of platelet concentration required to achieve optimal outcomes in male factor infertility cases, as well as incorporating supplementary long-term clinical outcomes, encompassing clinical pregnancy, live birth, miscarriage, and take-home baby rates. While our present research did not encompass the evaluation of embryo euploidy, previous evidence suggests that the morphological grading of euploid blastocysts correlates with implantation success and sustained pregnancy[38,39]. It is important to recognise that not all high-quality blastocysts are euploid, nor are all low-quality embryos aneuploid[40,41]. Consequently, measuring genetic normality remains

an important factor in determining long-term clinical success.

In conclusion, the present study shows that PRP serves as a promising therapeutic adjunct in sperm preparation protocols for ART, notably owing to its reliable enhancements in sperm motility and overall quality. Nevertheless, further research is required to standardise the preparation methodologies of PRP, definitively establish its clinical efficacy in larger, prospective trials, and thoroughly investigate its full therapeutic potential in the broader context of managing male factor infertility.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Authors' contributions

Binarwan Halim and Diana Novia contributed to the study's conceptualization, as well as reviewing and editing the manuscript. Yose E Hasibuan and Jesselyn Angellee were involved in the data analyses. The manuscript was written by Ivana G Nababan, Carine A Widjaja, and Jesselyn Angellee.

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