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(RESEARCH ARTICLE)

Comparative study of platelet-rich plasma in assisted reproductive technology: Impact on embryo quality and success rates

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Abstract

Objective: This study aimed to evaluate the impact of female-derived platelet-rich plasma (PRP) on embryo quality and success rates in assisted reproductive techniques (ART) through a prospective randomized controlled trial, with all procedures conducted following ethical registration and approval.

Methods: We used the PRP in ART procedures with gametes and embryos. We used intracytoplasmic sperm injection (ICSI) to fertilize the eggs. We conducted a RCT comparing a group treated with PRP to a control group from the same patient. This method helped us directly assess the impact of PRP on the quality of gametes and embryos. We performed a statistical analysis using SPSS to compare fertilization rates, blastocyst formation rates, and pregnancy outcomes between the two groups.

Results: The findings suggest that PRP treatment enhances embryo quality. The PRP group demonstrated significantly higher average fertilization rates (2.80 vs. 1.93 from 3.27 used in ICSI, $p = 0.004$) and blastulation rates (1.60 vs. 0.73, $p = 0.008$), as well as superior pregnancy and implantation rates (53% vs. 27%, $p = 0.027$; 40% vs. 18%, $p = 0.039$), indicating the potential effectiveness of PRP in improving outcomes in ART.

Conclusion: PRP in ART protocols enhances embryo quality and improves pregnancy outcomes. It offers a promising option for couples undergoing medical assisted procedures

Keywords: PRP; ART; ICSI; Embryo quality; Fertilization rates; Pregnancy outcomes

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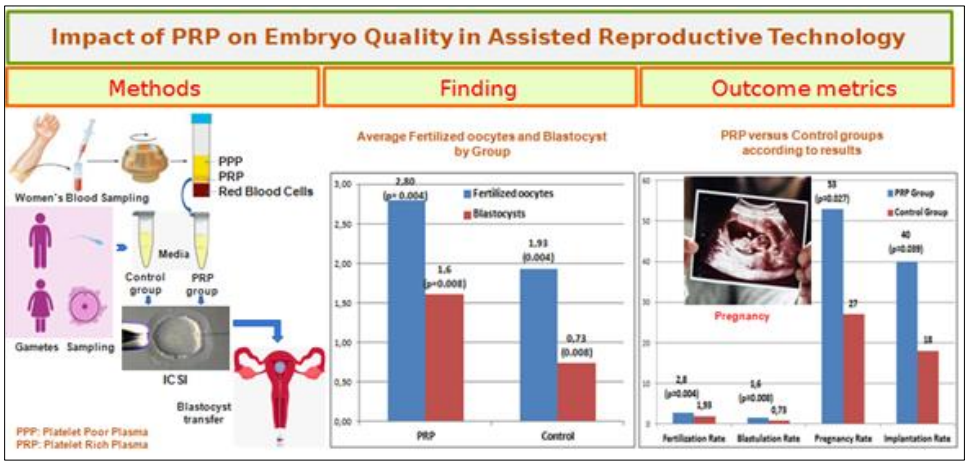
Graphical abstract



1. Introduction

Assisted reproductive technologies (ART) have significantly advanced the treatment options available to couples dealing with infertility, particularly in reproductive and post-reproductive health. A key focus within ART is improving embryo quality, often influenced by various maternal factors [1-3].

Platelet-rich plasma (PRP), derived from a woman's blood, represents a novel therapeutic approach in reproductive medicine. The ability of PRP to improve embryo quality offers a promising avenue for personalized treatment strategies aiming to improve reproductive outcomes [4-6].

This research compares PRP treatment to standard ART procedures, focusing on its effects on embryo quality and success rates. The goal is to determine if PRP can enhance ART outcomes and influence future reproductive medicine practices. Our study contributes to understanding reproductive health by exploring the potential of PRP to improve outcomes and shape future therapeutic approaches.

2. Methods

2.1. Context

This study explores the use of autologous platelet-rich plasma (PRP) in wash and culture media for assisted reproduction techniques (ART), conducted during the COVID-19 pandemic. The focus was on addressing logistical challenges and enhancing reproductive outcomes through personalized production and in-house manufacturing.

2.2. Study Design

This study focuses on supplementing wash and culture solutions for assisted reproductive methods (ART) with autologous platelet-rich plasma (PRP) during the COVID-19 pandemic. The main objective was to overcome operational hurdles and improve fertility outcomes by customizing production and developing in-house manufacturing capabilities.

2.3. Participant Recruitment

The study included couples with previous ART failures, with no additional costs incurred. The average age of the women was 33, with a 6-year age difference between partners. Exclusion criteria included cases of azoospermia and those with fewer than six oocytes.

2.4. Ethical Considerations

During the study, we scrupulously respected ethical standards. We duly informed all participants of the risks involved and the potential benefits. We gave them consent forms to sign, one for the collection of samples and the other specifically for the transfer of a single embryo from the PRP group.

2.5. Sample Collection

All women underwent hormone therapy to stimulate follicle growth and ovulation. Oocyte retrieval took place 36 hours after the treatment. The number of oocytes gathered from the follicular fluid differed based on the woman's age and their reaction to the therapy. Additionally, each woman contributed a blood sample for autologous PRP therapy, and male partners were mandated to provide a sperm sample through masturbation.

2.6. PRP Preparation

Blood was collected in citrate-containing tubes to prevent clotting and then subjected to centrifugation to separate the plasma. A second centrifugation was applied to create a concentrated solution of platelets, which was then combined with 1 milliliter of sterile saline solution and triggered with 0.02 milliliters of a 10% Calcium Gluconate solution. Then, the platelets were exposed to five immersions in liquid nitrogen to release their contents. Subsequently, the platelet lysate underwent filtration using 0.2-micrometer filters [7, 8].

2.7. Gamete and Embryo Treatment

We used a recommended and supplied medium by the Origio company to treat sperm, eggs, and embryos. In the control group, we followed the manufacturer's guidelines for all media. In the PRP group, we enhanced the culture media with 2% female-derived PRP, providing an optimal environment for our research. We achieved fertilization by applying the Intracytoplasmic sperm injection (ICSI). We divided the cohort of mature oocytes (in metaphase II) into two halves, one for the PRP group and one for the control group (CTR group). We transferred one blastocyst from the PRP group and cryopreserved the excess embryos.

2.8. Statistical Analysis

The research involved 15 ICSI attempts. We thoroughly examined the data using SPSS software to ensure accuracy and reliability. We assessed the normality of data distribution using the Shapiro-Wilk and Kolmogorov-Smirnov tests, and we compared the PRP group and the control group using the Wilcoxon Signed-Rank test. In light of mono-embryonic transfer from the PRP group, we utilized a binomial test to compare the pregnancy and implantation rates, using theoretical constants representing our statistical results from the previous series

3. Results

3.1. Qualitative Assessment

On the day of oocyte retrieval, cumulus-oocyte complexes (COCs) washed and cultured in a PRP-enriched medium displayed a more dispersed cellular structure. Oocytes in the PRP group prepared for microinjection exhibited a more uniform and smoother cytoplasm than the control group. By the first day post-retrieval, zygotes from the PRP group showed closer pronuclear proximity with more distinct nucleoli. By the fifth day, the PRP group had more blastocysts with advanced development, including higher-quality trophoblasts and inner cell masses. Despite these observations, the primary analysis focused on measurable outcomes.

3.2. Quantitative Assessment

3.2.1. Fertilization and Blastulation

Table 1 Descriptive Statistics Table

Variable	Group	Mean	Std. Deviation	Minimum	Maximum
Oocytes used in ICSI	PRP	3,27	0.884	2	5
	CTR	3,27	0.884	2	5
Fertilized Oocytes	PRP	2.80	0.775	2	4
	CTR	1.93	0.594	1	3
Blastocysts Formed	PRP	1.60	0.632	1	3
	CTR	0.73	0.704	0	2

In this case series, we examined the outcomes of fertilization and blastocyst formation between the PRP and control groups. On average, 3.27 mature oocytes in metaphase II were used for ICSI in each group (standard deviation = 0.884). The PRP group achieved an average of 2.80 fertilized oocytes (SD = 0.775), while the control group had a lower average of 1.93 fertilized oocytes (SD = 0.594). When it came to blastocyst formation, the PRP group also outperformed the control, with an average of 1.60 blastocysts (SD = 0.632) compared to 0.73 (SD = 0.704) in the control group (see Table 1).

Table 2 Tests of Normality

	Kolmogorov-Smirnov[a]			**Shapiro-Wilk**		
	Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
Fertilized oocytes with PRP	.249	15	.013	.806	15	.004
Fertilized oocytes in CTR group	.345	15	.000	.763	15	.001
Blastocystes formed with PRP	.295	15	.001	.761	15	.001
Blastocysts formed in CTR group	.251	15	.012	.798	15	.003

a. Lilliefors Significance Correction

For the normality tests, as in Table 2, the PRP group obtained p-values of 0.013 for fertilization and 0.004 for the Shapiro-Wilk test with the Kolmogorov-Smirnov test. Regarding blastocyst formation, both tests resulted in p-values of 0.001. On the other hand, the control group showed p-values of 0.000 for Kolmogorov-Smirnov and 0.001 for Shapiro-Wilk when it came to fertilization, along with p-values of 0.012 and 0.003, respectively, for blastocyst formation. Due to the non-normal distribution of the data, we conducted the Wilcoxon Signed-Rank test. The Z statistic was -2.919 with a p-value of 0.004 for fertilization and -2.648 with a p-value of 0.008 for blastocyst formation (see Table 3).

Table 3 Wilcoxon Signed Ranks Test Summary

Comparison	**Z-Statistic**	**p-Value**
Fertilized Oocytes (PRP vs. Control)	-2.919	0.004
Blastocysts Formed (PRP vs. Control)	-2.648	0.008

Graphical representations of the data distributions using histograms, Q-Q plots, and box plots in Fig. 1 (A and B) clearly illustrate the deviations from the normal distribution for fertilized oocytes (part A) and blastocyst formation (part B) in both groups (PRP and control).

3.2.2. Pregnancy and Implantation Rates

In this study, single-embryo transfers in the PRP group resulted in an average pregnancy rate of 53% (SD = 0.516) and an average implantation rate of 40% (SD = 0.507). The binomial test showed a one-tailed significance of 0.027 for the pregnancy rate compared to a hypothesized rate of 27% and a one-tailed significance of 0.039 for the implantation rate compared to a hypothesized rate of 18% (see Table 4).

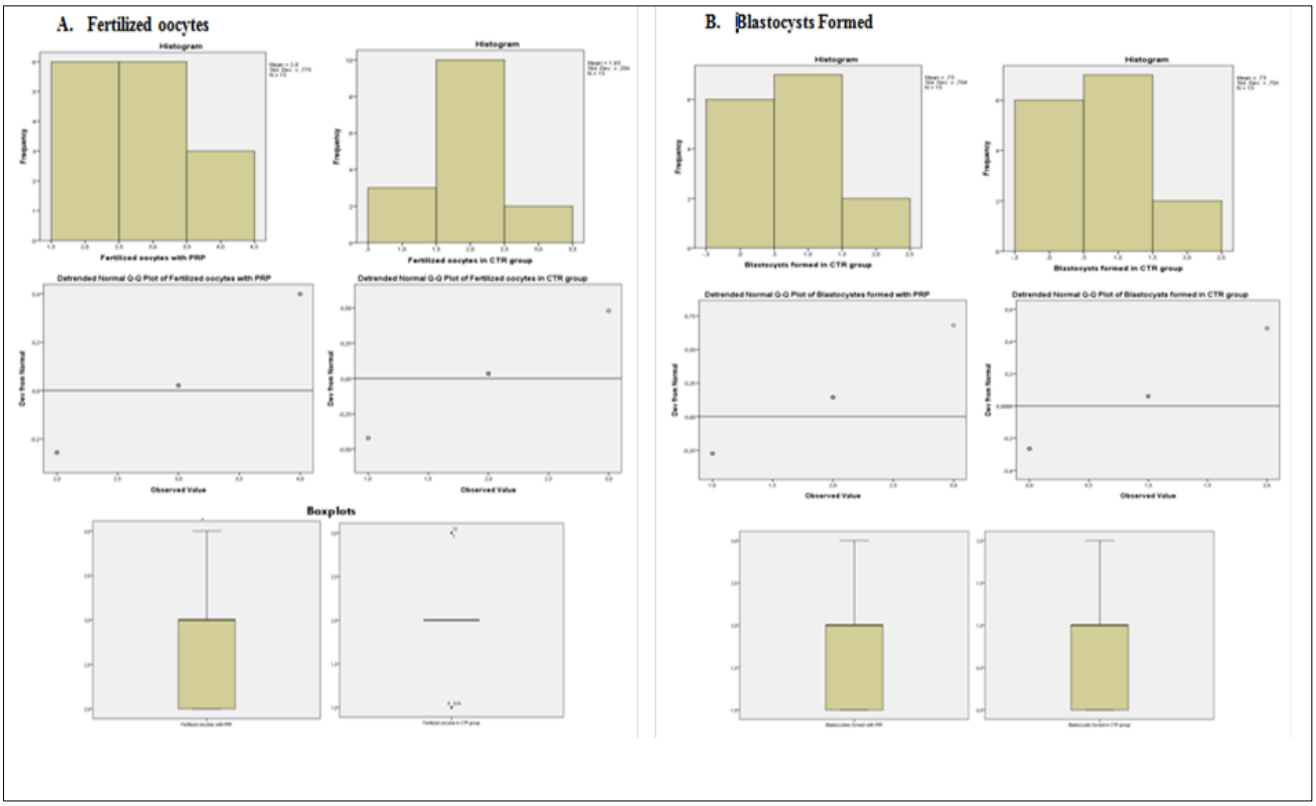


Figure 1 (A and B): Visual graphics with histograms, detrended normal Q-Q plots, and box plots. (A: Fertilized oocytes; B: Blastocysts formed)

Table 4 Binomial Test

		Category	N	Observed Prop.	Test Prop.	Exact Sig. (1-tailed)
Pregnancy with PRP	Group 1	Pregnancy	8	.53	.27	.027
	Group 2	No Pregnancy	7	.47		
	Total		15	1.00		
Implantation with PRP	Group 1	Implantation	6	.40	.18	.039
	Group 2	No Implantation	9	.60		
	Total		15	1.00		

Group 1: PRP Group; Group 2: CTR Group

4. Discussion

4.1. Statistical Analysis and Comparison with Existing Studies

The results obtained provide further evidence to previous research indicating that PRP stimulates the growth of cumulus and embryonic cells. Demonstrating its potential to enhance embryonic development both in vivo and in vitro, PRP ultimately makes it possible to improve assisted reproduction techniques and increase the chances of successful pregnancies [9-14].

Statistical analysis indicated significant differences between the PRP and control groups, particularly in fertilization and blastocyst formation rates. Normality tests revealed significant differences in favor of the PRP group, with p-values of 0.013 (Kolmogorov-Smirnov) and 0.004 (Shapiro-Wilk) versus 0.000 (Kolmogorov-Smirnov) and 0.001 (Shapiro-Wilk) for the control group. The Wilcoxon Signed-Rank test yielded a Z-statistic of -2.919 with a p-value of 0.004, confirming

the significant impact of PRP on fertilization rates. Similarly, blastocyst formation rates were significantly higher in the PRP group, with an average of 1.60 blastocysts (SD = 0.632) compared to 0.73 (SD = 0.704) in the control group. The Wilcoxon test results, with a Z-statistic of -2.648 and a p-value of 0.008, further validate these findings.

Additionally, the PRP group exhibited significantly higher pregnancy and implantation rates. The average pregnancy rate in the PRP group was 53% (SD = 0.516), with the binomial test showing a significant difference from the hypothesized rate of 27% (p = 0.027). The implantation rate was also significantly higher at 40% (SD = 0.507) than the hypothesized rate of 18% (p = 0.039). These results align with previous studies that suggest PRP's role in promoting embryonic development and improving pregnancy outcomes [10-15].

Overall, the data confirms the potential of PRP as a valuable adjunct in ART, enhancing both embryo quality and reproductive success. The significant improvements observed in fertilization, blastocyst formation, pregnancy, and implantation rates reinforce the promising role of PRP in assisted reproductive technologies.

4.2. How PRP works

PRP contains biologically active elements that regulate cellular growth and embryonic development signaling pathways, highlighting its significant potential in reproductive medicine [15-17].

PRP is rich in growth factors and cytokines, which can enhance the microenvironment of oocytes and embryos. Key growth factors in PRP, such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF), contribute to cellular proliferation, differentiation, and angiogenesis [17, 18]. These factors likely improve the quality of cumulus-oocyte complexes (COCs), enhance the development of zygotes, and support the formation of blastocysts. PRP contains key cytokines like interleukins (IL) and tumor necrosis factor (TNF), along with bioactive agents such as adenosine triphosphate (ATP), calcium, and zinc, which collectively boost natural growth factor production, enhance cell proliferation, and reduce oxidative stress, essential for efficient embryo development [19]. Additionally, PRP's mediators, antioxidants, progesterone, and other components synergistically improve sperm quality and reproductive potential, creating optimal conditions for embryo growth and maturation [19, 20]. The uniform, smoother cytoplasm observed in oocytes from the PRP group is attributable to the anti-apoptotic effects of the growth factors contained in PRP, which may reduce oxidative stress and enhance cytoplasmic maturation [19]. Additionally, the closer proximity of pronuclei and more distinct nucleoli in zygotes from the PRP group suggest enhanced nuclear maturation, which is crucial for early embryonic development. By promoting these cellular processes, PRP likely improves the overall quality of embryos, increasing their developmental potential and the likelihood of successful implantation.

4.3. Limitations and Clinical Relevance

The study suggests that Platelet-Rich Plasma (PRP) could significantly improve Assisted Reproductive Technology (ART) outcomes for couples who have not succeeded with traditional methods. PRP can increase fertilization and blastocyst formation rates, leading to higher pregnancy and implantation rates. However, limitations include a small sample size, varied preparation methods, and a lack of standardized protocols.

Further research, including larger-scale trials, is needed to confirm these findings and understand the mechanisms of PRP. If proven effective, PRP could offer hope to couples facing infertility challenges.

5. Conclusion

This case series highlights the significant benefits of integrating autologous PRP into ART procedures, particularly in improving fertilization, blastocyst formation, pregnancy, and implantation rates. This cost-effective solution can help alleviate the financial burden for couples undergoing multiple unsuccessful attempts. However, further large-scale controlled trials are essential to validate and explore the mechanisms underlying the positive impact of PRP on reproductive outcomes.

Compliance with ethical standards

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Statement of ethical approval

This comparative analysis focused on couples who experienced repeated failures with conventional ART. The research adhered to ethical guidelines and Moroccan law 47-14, which permits PRP therapy and in vitro procedures with informed consent. Ethical approval was not required, as the relevant ethics committee determined that informed consent from all participants was sufficient.

Statement of informed consent

All patients gave written informed consent.

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Authors Contributors

- Abdelkrim Salama contributed to conception and development of the study, acquisition of data, and manuscript writing and editing.
- Oumnia Bouaddi contributed to conception and development of the study, and reviewing drafts of the manuscript.
- Razana Zegrari contributed to contributed to conception and development of the study, and reviewing drafts of the manuscript.
- Ghyzlane EL Haddoumi contributed to the design and development of the study, and to the revision of preliminary versions of the manuscript.
- Jamal El Yazami contributed to the development of the study and to the care and follow-up of patients at Al Amal Fertility Center in Fez.
- Ilham kandoussi contributed to development of the study, interpretation of data and reviewing drafts of the manuscript.
- Azeddine Ibrahimi contributed to conception and development of the study, reviewing drafts of the manuscript and manuscript editing.

All authors have read and approved the final version of the manuscript. All authors take responsibility for all aspects of the reliability and unbiasedness of the data presented and their interpretation discussed.

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