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Efficacy of Platelet-Rich Plasma on Blastocyst Formation in ICSI Cycles Involving Low-Quality Sperm: A Randomized Study

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Study Protocol and Statistical Analysis Plan

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1. Background and Rationale

Platelet-rich plasma (PRP) is an autologous concentration of platelets containing growth factors that promote tissue regeneration and reduce oxidative stress. Poor sperm quality, particularly in severe oligoasthenoteratozoospermia (SOAT), is associated with impaired fertilisation, reduced embryo development, and lower blastocyst formation rates in intracytoplasmic sperm injection (ICSI) cycles.

Previous findings suggest that PRP improves sperm motility, morphology, and DNA integrity. However, its effect on downstream embryological outcomes remains insufficiently explored. This study aims to evaluate whether PRP incubation prior to ICSI improves blastocyst formation and embryo quality.

2. Objectives

Primary Objective:

To evaluate the effect of PRP incubation on blastocyst formation rate in ICSI cycles involving low-quality sperm

Secondary Objectives:

- To assess fertilisation rate
- To assess cleavage rate
- To evaluate blastocyst quality (good, fair, poor)

3. Study Design

This study is a prospective, randomized controlled study conducted at Halim Fertility Center, Stella Maris Women’s and Children’s Hospital, Medan, Indonesia, between January 2023 and August 2023.

Participants were recruited using consecutive sampling. Couples undergoing more than one IVF-ICSI cycle were excluded.

4. Participants

4.1 Inclusion Criteria (Male)

- Sperm concentration <5 million/mL
- Total motility <42%
- Normal morphology <4%
- Provided informed consent

4.2 Exclusion Criteria (Male)

- Leukospermia
- Reproductive infections (HIV, hepatitis)
- Recent drug exposure (within 3 months)
- Fever within 3 months
- Testicular carcinoma
- Use of second ejaculate or cryopreserved sperm
- Retrograde ejaculation
- Coagulopathy

4.3 Exclusion Criteria (Female)

- Age ≥40 years
- Polycystic ovarian syndrome (Rotterdam criteria)
- Low ovarian reserve (AFC <5 or AMH <0.5–1.1 ng/mL)

5. Randomisation and Blinding

Participants were randomly allocated into two groups using registration number parity:

Odd numbers: PRP group

Even numbers: Control group

Embryologists performing ICSI and outcome assessments were blinded to group allocation.

6. Interventions

6.1 PRP Group

Sperm samples were incubated with autologous PRP for one hour prior to ICSI. PRP was prepared from 10 mL peripheral blood using a two-step centrifugation process. A ratio of 30 µL PRP to 0.3 mL sperm (10% v/v) was used.

6.2 Control Group

Standard sperm preparation without PRP incubation.

All samples underwent density gradient centrifugation prior to intervention.

7. Ovarian Stimulation and ICSI Procedure

Female partners underwent controlled ovarian stimulation using a GnRH antagonist protocol. Oocyte retrieval was performed 35–36 hours after hCG trigger. Mature oocytes (MII) were inseminated using ICSI 38–42 hours post-trigger.

8. Embryo Culture and Assessment

Fertilisation assessed at 17±1 hours (2PN). Cleavage assessed on day 3. Blastocyst assessed on day 5.

Blastocysts were graded using the Gardner classification:

Good: AA

Fair: AB, BA, BB

Poor: AC, CA, BC, CB, CC

All assessments were performed by a single blinded embryologist.

9. Outcome Measures

Primary Outcome:

Blastocyst formation rate

Secondary Outcomes:

Fertilisation rate

Cleavage rate

Blastocyst quality

10. Statistical Analysis Plan

Statistical analysis was conducted using SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

Normality assessed using Shapiro–Wilk test

Continuous variables analysed using Student’s t-test or Mann–Whitney U test

Categorical variables analysed using Chi-squared test

Data presented as mean ± standard deviation (SD) or median (IQR)

Categorical data presented as percentages (%)

Statistical significance set at $P < 0.05$

No adjustment for multiple comparisons was performed as outcomes were pre-specified.

11. Ethical Considerations

The study was approved by the Institutional Ethics Committee of Stella Maris Women's and Children's Hospital (Approval No. 608.1/Dir/RSIA.SM/XII/2022; approved on 19 December 2022).

Written informed consent was obtained from all participants prior to enrolment.

12. Data Confidentiality

All participant data were anonymized and handled in accordance with ethical and regulatory standards. No identifiable personal data are included in this document.