

STUDY PROTOCOL

Official Title: Optimal Allogenic Platelet-rich Plasma Concentration for Oocyte In Vitro Maturation in Women With PCOS

Unique Protocol ID: PRP-PCOS-COHORT-UGM

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This study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (protocol number: KE/FK/0022/EC/2023, released January 5, 2023). A total of 34 germinal vesicle (GV) stage oocytes exhibiting normal morphology were retrieved via the ovum pick-up (OPU) procedure from 15 women diagnosed with PCOS undergoing IVF treatment at Permata Hati Infertility Clinic between 2023 and 2024. An initial pilot experiment tested three different concentrations of PRP supplementation (5%, 25%, and 50%) using three GV-stage oocytes per group. The concentration that provided the highest maturation rate while maintaining the quality and stability of the culture medium was selected as optimal.

Following this, the remaining 28 GV-stage oocytes were randomly allocated into two groups: a control group receiving no PRP (0%, n=14) and a treatment group supplemented with PRP at the optimal concentration (5%, n=14). Prior to evaluation, cumulus-oocyte complexes (COCs) were enzymatically denuded with hyaluronidase (Vitrolife, Sweden) and mechanically cleaned using micropipettes (170 μ m and 140 μ m) to remove cumulus cells, enabling direct assessment of PRP effects on oocytes. Each oocyte was then cultured for 24 hours at 37°C in either control media (G-IVF™) or treatment media containing PRP at one of the three tested concentrations.

The primary outcome was oocyte maturation, defined by the transition from GV to MII stage, determined by the presence of the first polar body after 24 hours. Secondary outcomes included total oocyte score (TOS) and fertilization outcomes. TOS is a semi-quantitative morphological assessment of oocyte quality, based on six parameters: oocyte shape, size, ooplasm characteristics, perivitelline space, zona pellucida thickness, and polar body morphology. Each parameter was scored on a standardized three-point scale (+1, 0, -1) according to the system by Lazzaroni-Tealdi [14]. Raw TOS data and scoring criteria are available in Mendeley Data. Fertilization was evaluated approximately 17 ± 1 hours post-injection, consistent with standard pronuclear assessment for ICSI-derived zygotes, with fertilization defined by the presence of two pronuclei (2PN) and two polar bodies under microscopy.

PRP and Culture Media Preparation

To reduce confounding factors, allogenic PRP was utilized. A healthy female donor aged 20–30 years, with a history of spontaneous pregnancy and no reproductive disorders, was selected. Blood (35 mL) was collected and centrifuged sequentially at $103 \times g$ for 10 minutes and $230 \times g$ for 15 minutes, yielding approximately 12 mL of PRP, which was aliquoted into 1.5 mL microtubes and stored at -80°C until use. PRP preparation followed routine clinical procedures; however, details such as platelet and leukocyte counts or growth factor quantification were not recorded.

Oocyte maturation was conducted in G-IVF PLUS medium (Vitrolife, Gothenburg, Sweden). PRP stock solutions were prepared at 5%, 25%, and 50% concentrations. Frozen aliquots were thawed at room temperature for 10 minutes prior to mixing with the culture medium, and remaining stocks were kept frozen until required.

Statistical Analysis

Maturation outcomes were analyzed using Fisher's exact test in IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA), with statistical significance defined as $p < 0.05$. This method was chosen due to the small sample size and comparable variance among groups. Because of unequal MII oocyte counts across groups, TOS analysis was not performed statistically to avoid bias from sample size imbalance; TOS results are presented descriptively.