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RESEARCH ARTICLE

Effect of Autologous Platelet-Rich Plasma (PRP) on Endometrial Thickness of Wistar Rats Under Antagonist Protocol in In Vitro Fertilization

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ABSTRACT

Introduction: **The advancement of Assisted Reproductive Technology (ART) has significantly improved fertility outcomes, particularly through In Vitro Fertilization (IVF). One critical factor influencing IVF success is the health and receptivity of the endometrium. This study aimed to evaluate the effect of Autologous Platelet-Rich Plasma (PRP) on endometrial thickness in Wistar rats subjected to an antagonist protocol during IVF.**

Methods: **This experimental study used a randomized post-test only controlled group design involving 40 Wistar rats, divided into two groups: the treatment group (administered PRP) and the control group (no PRP administration). Endometrial thickness was measured using histomorphometry, and the results were analyzed using the Mann-Whitney test.**

Result: **A total of 40 rats were included in the study. PRP administration significantly increased endometrial thickness in rats subjected to the IVF antagonist protocol compared to the control group ($p < 0.001$). The mean endometrial thickness in the treatment group was 335.5 ± 32.2 μm, while in the control group, it was 228.5 ± 42.7 μm.**

Conclusion: **Autologous Platelet-Rich Plasma (PRP) significantly increases endometrial thickness in Wistar rats under the IVF antagonist protocol, suggesting its potential in enhancing IVF success. This finding has important implications for the field of reproductive medicine.**

Keywords: Autologous Platelet-Rich Plasma, endometrial thickness, in vitro fertilization.

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1. Introduction

The development of Assisted Reproductive Therapy (ART), especially with the introduction of In Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI), has shown significant progress over the years. IVF is the most common form of ART, where oocytes are fertilized in vitro, meaning outside the body, and then transferred to the uterus to develop into a pregnancy [1]. The first successful live birth from IVF was reported by Robert Edwards, Ph.D., and Patrick Steptoe, MD, in 1978, an achievement that earned Dr. Edwards the Nobel Prize in 2010 [2]. IVF success is influenced by factors such as age, embryo quality, and endometrial receptivity [3]. The use of Gonadotropin-Releasing Hormone (GnRH) antagonist protocols in ovarian stimulation has been shown to reduce the risk of ovarian hyperstimulation syndrome (OHSS) [4]. In 2018, the CDC reported IVF success rates ranging from 50.7% for women under 35 to 7.9% for women over 40 [5], while Indonesia reported a success rate of around 29% in 2017 [6]. Some studies suggest that GnRH antagonist protocols can reduce endometrial thickness, a key indicator of endometrial receptivity [7], [8].

Platelet-rich plasma (PRP), which contains numerous growth factors and cytokines, has been proposed as a therapy to enhance endometrial thickness in patients undergoing IVF with GnRH antagonist protocols. Autologous PRP, derived from the patient's own blood, may help restore endometrial thickness by improving estrogen receptor regulation in the endometrium [8]. Although PRP has been applied in human studies internationally, ethical and legal constraints in Indonesia have limited such trials to animal models, such as Wistar rats. This study aims to

investigate the effects of autologous PRP on endometrial thickness in Wistar rats treated with a GnRH antagonist protocol.

2. Materials and Methods

2.1. Study Design

This study employed a randomized post-test only controlled group design to assess the effect of Autologous Platelet-Rich Plasma (PRP) on endometrial thickness in Wistar rats. The rats were divided into two groups: a treatment group receiving PRP and a control group with no PRP administration.

2.2. Sample Size and Sampling Technique

A total of 40 Wistar rats were included in the study, with 20 rats allocated to each group. Samples were selected using a simple random sampling technique.

2.3. Experimental Procedures

In the treatment group, Autologous PRP was prepared by collecting blood from the rats, which was then processed to isolate PRP through centrifugation. PRP was administered to the treatment group, while the control group received no intervention. Both groups were subjected to a GnRH antagonist protocol in an IVF simulation. Endometrial thickness was measured after the PRP administration using histomorphometric analysis.

2.4. Outcome Measurement

Endometrial thickness was measured by histomorphometry, and data were collected post-intervention. The thickness of the endometrium was recorded for both the treatment and control groups.

2.5. Data Analysis

Data were analyzed using the Mann-Whitney U test to determine the statistical significance of differences between the two groups. A p-value of less than 0.05 was considered statistically significant.

2.6. Ethical Considerations

All experimental procedures involving animals were conducted following the ethical guidelines and were approved by the Animal Care and Use Committee (ACUC).

3. Results

This experimental study utilized a randomized posttest-only controlled group design, involving 40 Wistar rats divided into two groups: 20 rats received autologous PRP as the treatment group, while 20 rats were assigned to the control group. The research was conducted at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, Denpasar. Rats aged 8–12 weeks with regular estrous cycles and weighing 200–230 grams were used. A simple random sampling technique was applied to select the sample from the available 47 rats at the Pharmacology Laboratory. Using Microsoft Excel's random number generator, rats numbered 1–20 were placed in the treatment group and received PRP, while rats numbered 21–40 were placed in the control group without PRP.

Approval for the study was obtained from the Integrated Biomedical Laboratory Unit. The study's results were presented as follows: the mean age of rats in the treatment group was 62.95 ± 1.31 days, and in the control group, it was 62.45 ± 1.60 days. There was no significant difference in the mean age between the two groups ($p = 0.288$). The mean initial body weight for the treatment group was 211.24 ± 1.73 grams, while for the control group, it was 211.85 ± 1.61 grams, with no significant difference between the groups ($p = 0.257$). By the end of the study, the mean body weight was 227.69 ± 1.36 grams for the treatment group and 228.30 ± 1.26 grams for the control group, with no significant difference ($p = 0.152$; see Table I).

Regarding the effect of autologous PRP on endometrial thickness, endometrial samples from both groups were examined using an Olympus CX41 microscope and OptilabPro camera. Images were analyzed using ImageRaster software to measure endometrial thickness (Fig. 1) in four directions (12, 3, 6, 9 o'clock positions). Since the endometrial thickness data from the control group were not normally distributed, the Mann-Whitney test was used for comparison.

Histological examination revealed that the mean endometrial thickness in the treatment group was 33.55 ± 3.22 μm, significantly higher than the control group's 22.85 ± 4.27 μm ($p < 0.001$). This significant difference indicates that autologous PRP administration increases endometrial thickness in rats subjected to ovarian stimulation with a

TABLE I: Demographic Characteristics of Treatment and Control Groups

Characteristic	Treatment group (N = 20)		Control group (N = 20)		P-value
	Mean	SD	Mean	SD	
Age (days)	62.95	1.31	62.45	1.60	0.288
Initial body weight (grams)	211.24	1.73	211.85	1.61	0.257
Final body weight (grams)	227.69	1.36	228.30	1.26	0.152

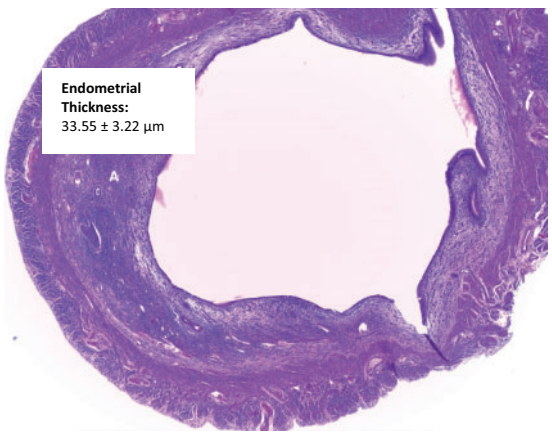

Fig. 1. Histological image of endometrial thickness (A = endometrium) in Wistar rats from the treatment group.

TABLE II: Endometrial Thickness Comparison Between Treatment and Control Groups

Mean	Treatment group (n = 20)		Control group (n = 20)		U
	Mean	SD	Mean	SD	
Endometrial thickness (μm)	33.55	3.22	22.85	4.27	<0.001

GnRH antagonist protocol compared to those without PRP ($p < 0.05$; Table II).

4. Discussion

The failure of IVF therapy can have significant financial, psychological, and mental health impacts on patients and their partners. Several factors influencing IVF success include age, sperm quality, embryo quality, the number of embryos transferred, and endometrial thickness [9]. Endometrial thickness affects receptivity, and studies show that thickness during fresh oocyte IVF cycles is critical for success [10]. A thickness of 7–10 mm is associated with better IVF outcomes [4], [11], and PRP is considered a safe alternative to enhance endometrial thickness. Wistar rats were chosen for this study due to their short gestation period, allowing transgenerational analysis [12], [13]. Optimal reproductive age for rats is between 28 and 42 days [14], [15], and the rats used met this criterion. There was no significant difference in body weight between groups during the study, reflecting healthy conditions in both groups.

PRP has been studied in animal models, with Kim *et al*. [16] demonstrating that intrauterine PRP reduced fibrosis and increased cell proliferation. Schalich *et al*. [17] found PRP increased cell proliferation and anti-inflammatory factors in bovine endometrial cells. GnRH antagonists are widely used in ART, but their use in fresh embryo transfer can reduce implantation rates by thinning the endometrium [8]. PRP, known to promote cell proliferation and tissue regeneration through growth factor release [18], is considered safer as it uses autologous blood. PRP has been used in wound healing, bone regeneration, and skin rejuvenation [19], and it enhances the implantation environment in cases of recurrent implantation failure. Sildenafil shows promise but lacks sufficient RCT evidence, while studies from Japan suggest PRP is more effective [20].

In 2018, Molina *et al*. [21] reported that PRP increased endometrial thickness to >7 mm after the first treatment and >9 mm after the second in patients with thin endometrium undergoing IVF. This was supported by other studies, showing significant increases in thickness and pregnancy rates with PRP [11]. PRP administration via infusion and injection both increased thicknesses, though injection was more effective [22]. In this study, the treatment group's endometrial thickness was significantly higher (33.55 ± 3.22 μm) than the control group's (22.85 ± 4.27 μm; $p < 0.001$), supporting the therapeutic potential of autologous PRP in enhancing endometrial thickness and improving IVF outcomes in cases of thin endometrium.

5. Conclusion

The study concluded that the mean endometrial thickness in the treatment group was 33.55 ± 3.22 μm, significantly higher than the control group's mean of 22.85 ± 4.27 μm, with a highly significant p-value below 0.001. These results suggest that PRP administration led to a substantial increase in endometrial thickness compared to the control group. This demonstrates the efficacy of PRP in enhancing endometrial receptivity in the context of a GnRH antagonist protocol.

The positive results from this study using Wistar rats suggest that PRP can improve endometrial thickness, paving the way for human trials in Indonesia. Given that international studies have already explored the use of PRP in human IVF treatment, it is now time for Indonesia to contribute to the global scientific community by conducting human research. This could provide further insights into the potential of PRP as a therapeutic option to address issues of thin endometrium and IVF failure, thereby advancing the current understanding and management of reproductive health challenges.

Author Contributions

Anak Agung Gde Kiki Sanjaya Dharma conceptualized the study and designed the research framework. Anak Agung Ngurah Anantasika and Anom Suardika supervised the experimental procedures and contributed to the manuscript's revision. Carried out animal experiments and data collection. Data analysis was performed by Anak Agung Gde Kiki Sanjaya Dharma. All authors contributed to the drafting and finalization of the manuscript and approved the final version for submission.

Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this study. No financial relationships or external influences have affected the objectivity in conducting the research or interpreting the results.

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