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Preliminary Report of the Effects of Platelet-Rich Plasma on the Ovarian Function and Oocyte Quality in Women with Ovarian Failure

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ABSTRACT

This study investigated the effects of platelet-rich plasma (PRP) treatment on ovarian function and pregnancy outcomes in three patients with severe reduction of ovarian reserve. The patients had previously undergone multiple cycles of assisted reproductive technology (ART) without favorable results. Prior to PRP treatment, follicle-stimulating hormone (FSH), estradiol (E2), and anti-Mullerian hormone (AMH) levels were assessed. PRP was administered through intraovarian injection. Hormonal assessments were performed at 6–7 months after the first PRP injection. The results showed major improvements in ovarian function, as evidenced by decreased FSH levels and increased AMH levels in all three patients. The number of mature oocytes retrieved per cycle and the ovum-to-blastocyst rate were also improved. All the patients achieved successful pregnancies and gave birth to healthy infants. These findings suggest that PRP may enhance ovarian function, oocyte quality, and pregnancy outcomes in patients with severely reduced ovarian reserve. Further research is necessary to validate these results and explore the underlying mechanisms.

Keywords: Platelet-Rich Plasma; Pregnancy Outcome; Reproductive Techniques; Assisted; Ovarian Reserve; Infertility.

INTRODUCTION

The quality of ovarian function and oocytes plays a critical role in achieving successful pregnancy rates. Infertility has emerged as a significant global issue over the past few decades, affecting approximately 12% of the global population. Despite overall population growth, infertility remains a prevalent concern. One of the primary factors contributing to infertility is diminished ovarian function, which strongly influences oocyte quality. Poor oocyte quality significantly reduces the chances of successful implantation, underscoring the importance of ovarian function in infertility treatment.

Women with decreased ovarian function face reduced probabilities of natural pregnancy or successful pregnancy through in vitro fertilization (IVF). This condition encompasses premature ovarian insufficiency (POI), premature ovarian failure (POF), and poor ovarian response (POR). POI and POF occur in patients under the age of 40 and significantly decrease the chances of successful conception. POR is associated with aging ovaries. In summary, decreased ovarian function adversely affects the outcomes of natural pregnancy as well as IVF. Despite advancements in infertility treatment, effective solutions for decreased ovarian function remain elusive. Overcoming the challenges associated with decreased ovarian function requires novel approaches and therapeutic interventions.

Platelet-rich plasma (PRP) has emerged as a potential treatment option due to its ability to restore ovarian function (Farimani et al., 2019; Pantos et al., 2019; Sills et al., 2018). PRP serves as a reservoir of growth factors, containing a rich concentration of regenerative proteins and cytokines (Amable et al., 2013; Masuki et al., 2016). Widely used in joint and ligament repairs (Andia et al., 2012; Paoloni et al., 2011), PRP has also shown promise in improving endometrial thickness and enhancing pregnancy outcomes in IVF (Kim et al., 2022; Nazari et al., 2022; Xu et al., 2022). It has also been reported that PRP optimized the endometrial environment and ultimately improved receptivity (Mouanness et al., 2021; Suzuki et al., 2023). Additionally, studies have suggested that PRP may restore anti-Mullerian hormone (AMH) levels, ultimately improving ovarian function (Aflatoonian et al., 2021; Sfakianoudis et al., 2020), and also the qualities of the oocyte and the embryo (Parvanov et al., 2022). Furthermore, PRP has demonstrated the potential to enhance oocyte quality when cocultured with oocytes (Moulavi et al., 2020). PRP is enriched with a high concentration of growth factors. Due to this characteristic, PRP has emerged as a promising therapeutic approach for addressing decreased ovarian function.

This study, to our knowledge, is the first Japanese report demonstrating the potential of PRP to improve ovarian function and aimed to evaluate the impact of intraovarian PRP injections

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on ovarian function and oocyte quality in women with ovarian failure. Three cases from Maruta ART Clinic in Nagoya, Japan, between 2021 and 2022 are reported, wherein each patient received intraovarian PRP injections. The results showed increased AMH levels following PRP injections in all three patients. Furthermore, two patients exhibited improved ovum-to-blastocyst rates. Based on these findings, PRP demonstrated the potential to improve ovarian function, and subsequently enhance oocyte quality in patients with ovarian failure. Although PRP appeared to be a safe treatment option, further investigations are necessary to fully explore its potential in treating ovarian failure.

CASE REPORT

Patient description

During the initial consultation appointment, detailed reproductive histories were recorded for all three patients in accordance with the clinic's protocol. Prior to initiating PRP treatment and at 6 to 7 months after the first PRP injection, their hormonal profiles were assessed. Levels of follicle-stimulating hormone (FSH), estradiol (E2), and AMH were determined on an unspecified cycle day prior to PRP treatment.

PRP preparation

PRP was prepared as described previously (Suzuki et al., 2023). Briefly, a total of 20 mL of peripheral blood was collected from each patient using Acti-PRP tubes (Aeon Biotherapeutics Corp., Taipei, Taiwan). The tubes were then centrifuged at 3600 rpm for 6 minutes. Following centrifugation, 1 mL of plasma remained in the tubes, and the buffy coat above the separation gel was removed and thoroughly mixed. Subsequently, a total of 1–2 mL of PRP was collected using a syringe and prepared for injection.

Intraovarian injection of PRP

PRP injections were administered 10 days after the start of menstrual bleeding. Under minimal sedation and transvaginal ultrasound monitoring, a multifocal intramedullary infusion of approximately 1 mL PRP was performed into each ovary using a 19-gauge single-lumen needle. Following PRP treatment, all three patients underwent controlled ovarian stimulation using clomiphene citrate (CC) + FSH/human menopausal gonadotropin (hMG) + gonadotropin-releasing hormone (GnRH) antagonist. Ovarian monitoring was performed, and ovulation was triggered with an injection of 250 µg recombinant human chorionic gonadotropin (hCG) (Ovidrel™ Merck Serono S.A., Switzerland). Oocytes were retrieved via the transvaginal route under ultrasound guidance. Mature oocytes were fertilized through IVF or intracytoplasmic sperm injection (ICSI), and the resulting embryos were cultured and subsequently cryopreserved at either the cleavage stage or the blastocyst stage. The quality of blastocysts was evaluated according to Gardner's criteria (Gardner and Sakkas, 2003).

Hormonal assessment and follow-up

Ovarian function was assessed through hormonal measurements for all patients. Serum AMH and FSH levels were evaluated before PRP injection and at 6 months after the first PRP injection. The Elecsys® AMH assay and the Elecsys® FSH assay on a Cobas e411 analyzer (Roche Diagnostics Japan, Japan) were used to determine AMH and FSH levels, respectively. The focus of the study was on the process of egg retrieval until an adequate number of frozen embryos were obtained. Hormone replacement therapy (HRT) and frozen embryo transfer (FET) cycles were performed after a period of rest, depending on the patients' condition and preferences. Ten days after embryo transfer, β-hCG tests were conducted, and clinical pregnancy was defined by the detection of fetal heart activity. Transvaginal ultrasonography was performed 5 weeks after a positive β-hCG test. All three patients were followed up for 1 year after the first PRP injection to assess pregnancy outcomes, and the pregnant cases were monitored until delivery.

Case 1

Patient 1, aged 39, had previously undergone 3 years of infertility treatments due to her husband's intravaginal ejaculation disorder. Following two cycles of CC + FSH/hMG + GnRH antagonist treatment, the patient underwent oocyte retrieval resulting in one immature, one degenerated, and seven MII oocytes. ICSI was performed, resulting in the development of six 2PN embryos and one 1PN embryo. Subsequently, two cleavage-stage embryos and three blastocysts were obtained and cryopreserved. The average number of matured oocytes per retrieval was 3.50 (7/2), and the ovum-to-blastocyst rate was 42.86% (3/7) (Table 1). HRT and embryo transfer were conducted, resulting in a positive β-hCG test, but no heartbeat was detected at 7 weeks. The patient underwent three additional embryo transfers, all of which were negative. Intraovarian PRP injection was then performed with the patient's consent. Prior to PRP administration, the FSH level was 12.06 mIU/mL and AMH was 0.46 ng/mL (Table 1).

Three months after the PRP injection, FSH levels decreased to 6.03 mIU/mL, and AMH increased to 0.70 ng/mL in Patient 1 (Table 1). FSH decreased by 50%, while AMH increased by 52.17%. These major changes suggested improvements in ovarian function. Four consecutive retrievals yielded a total of 17 MII oocytes, which were all fertilized and cultured to the blastocyst stage. Eleven excellent-quality blastocysts were obtained. The average number of matured oocytes per retrieval was 4.25 (17/4), and the ovum-to-blastocyst rate was 64.71% (11/17) (Table 1). Both the number of matured oocytes per retrieval and the ovum-to-blastocyst rate increased by 0.75 oocytes and 50.98%, respectively, which suggested that PRP improved oocyte quality. Following HRT, one frozen embryo was transferred, resulting in a negative biochemical pregnancy test. In the subsequent cycle, two frozen embryos were

Table 1. AMH, FSH levels, and ovum-to-blastocyst rate before and after PRP injection.

		Before PRP treatment				After PRP treatment			
Case	Age (year)	AMH (ng/mL)	FSH (ng/mL)	Matured oocytes per retrieval	Ovum-to-blastocyst rate (%)	AMH (ng/mL)	FSH (mIU/mL)	Matured oocytes per retrieval	Ovum-to-blastocyst rate (%)
1	39	0.46	12.06	3.50	42.86	0.70	6.03	4.25	64.71
2	39	0.62	18.99	3.25	46.15	1.14	7.86	2.43	41.18
3	42	0.06	29.12	1.00	36.00	0.24	22.33	1.31	41.18

transferred, resulting in a positive biochemical pregnancy test and a healthy pregnancy without complications. A healthy male infant weighing 2604 g was born at 38 weeks of gestation.

Case 2

Patient 2, aged 39, had undergone 2 years of infertility treatments and failed IVF attempts before seeking treatment at our clinic at the age of 36. The patient's FSH level was 5.40 mIU/mL and AMH level was 1.38 ng/mL. Despite narrow fallopian tubes observed during uterine tubal radiography, two cycles using timed intercourse were attempted but were unsuccessful. The patient opted for ART treatment. After four cycles of CC + FSH/hMG + GnRH antagonist, a total of 18 oocytes were retrieved, with 13 being MII mature oocytes. IVF resulted in the cryopreservation of six blastocysts graded as 4BB, 4AB, and 5AB. The average number of oocytes retrieved per retrieval was 3.25 (13/4), and the ovum-to-blastocyst rate was 46.15% (6/13) (Table 1). Subsequently, three HRT cycles were conducted for embryo transfer, but all β-hCG tests were negative. In consultation with the patient, PRP treatment was considered and approved. Before PRP injection, the FSH level was 18.99 mIU/mL and AMH was 0.62 ng/mL (Table 1).

Seven months after PRP administration, the FSH level decreased to 7.86 mIU/mL and AMH increased to 1.14 ng/mL (Table 1). The rate of decrease in FSH was 58.61%, while the rate of increase in AMH was 83.87%, suggesting improved ovarian function. Over seven consecutive cycles, a total of 17 oocytes were retrieved, all of which were mature MII oocytes and successfully fertilized. Seven high-quality blastocysts were obtained and graded as 5AB, 4AB, and 5BB. The average number of oocytes per retrieval was 2.43 (17/7), and the ovum-to-blastocyst rate was 41.18% (7/17) (Table 1). One frozen embryo was transferred in an HRT cycle, resulting in a negative biochemical pregnancy test. In the following cycle, another frozen embryo was transferred, leading to a positive biochemical pregnancy test and a healthy pregnancy without complications. A healthy boy weighing 3015 g was born via planned cesarean section at 38 weeks of pregnancy due to breech presentation.

Case 3

Patient 3, aged 42, had been experiencing infertility for 5 years since the age of 37. After three failed artificial insemination attempts, the patient opted for further treatment. The patient's AMH level was 0.21 ng/mL and FSH was 28.60 mIU/mL. A hysterosalpingogram (HSG) revealed right tubal swelling and left tubal obstruction, leading to laparoscopic tubal formation surgery to correct the left tubal obstruction. A total of 27 oocytes were retrieved in 25 retrieval cycles, resulting in 25 MII mature oocytes. Three cleavage-stage embryos and nine blastocysts were cryopreserved. On average, one (25/25) matured MII oocyte was retrieved per oocyte retrieval, and the ovum-to-blastocyst rate was 36% (9/25) (Table 1). Despite multiple oocyte retrievals and transfers, the patient did not conceive. Intraovarian PRP injection was considered and approved by the patient. Before PRP treatment, the FSH level was 29.12 mIU/mL and AMH level decreased to 0.06 ng/mL (Table 1).

The AMH level increased to 0.24 ng/mL and FSH decreased to 22.33 mIU/mL 4 months after PRP injection (Table 1). The AMH level increased by threefold, while the FSH level decreased by 0.23-fold, suggesting improvement in ovarian function. After 13 consecutive ovulation stimulation cycles following intraovarian PRP injection, 19 oocytes were retrieved, with 17 undergoing ICSI. Seven blastocysts were obtained and graded as 5AB, 5BB, 5BC, 4CC, and 5CC, which were subsequently cryopreserved. On average, 1.31 (17/13) matured oocytes were retrieved per oocyte retrieval, and the ovum-to-blastocyst rate was 41.18% (7/17) (Table 1). Both the number of matured oocytes per retrieval and the ovum-to-blastocyst rate increased by 0.31 oocytes and 14.39%, respectively, following PRP treatment, indicating improved oocyte quality. A 5AB blastocyst was transferred on Day 6 of the HRT cycle, resulting in a positive pregnancy test. The patient underwent an emergency cesarean section at 34 weeks and 2 days, and a 1629-g girl was born. The baby's weight increased to 2120 g, and was discharged safely.

DISCUSSION

This is the first Japanese study investigating the use of intraovarian PRP for improving ovarian function. The results demonstrate that intraovarian PRP injections have the potential to improve ovarian function and enhance oocyte quality in women with ovarian failure. Ovarian failure, characterized by decreased ovarian function and diminished oocyte quality, is a significant contributor to infertility. Despite advances in infertility treatments, effective solutions for decreased ovarian function remain limited.

In this study, three cases of women with ovarian failure were treated with intraovarian PRP injections, and the outcomes were evaluated. The results showed an increase in AMH levels following PRP injections in all three patients, suggesting an improvement in ovarian function. AMH is an essential marker of ovarian reserve and reflects the pool of follicles available for potential ovulation. The increase in AMH levels suggests that PRP may help restore and stimulate ovarian follicle development, potentially leading to better oocyte quality. Moreover, two out of the three patients exhibited improved ovum to blastocyst rates following PRP treatment. This increase in blastocyst formation suggests that PRP may positively influence oocyte maturation and embryo development, ultimately leading to improved pregnancy outcomes. In addition to the improvement in ovarian function and oocyte quality, the study also reported successful pregnancies resulting in the birth of healthy infants in three patients after intraovarian PRP treatment.

PRP has been explored for its regenerative properties in various medical fields, including orthopedics and wound healing (Andia et al., 2012; Paoloni et al., 2011). In recent years, studies have shown its positive effects on endometrial thickness, endometrial receptivity, and pregnancy outcomes in IVF treatments (Lin et al., 2023). The observed effect of PRP to optimize the endometrial environment may enhance embryo implantation, making it a promising adjunct therapy for infertility treatment (Suzuki et al., 2023). Various studies have demonstrated that PRP may stimulate ovarian tissue regeneration and repair (Ahmadian et al., 2020; Allam et al., 2022). Some studies have reported increased AMH levels following PRP treatment, indicating improved ovarian reserve and function (Pantos et al., 2019). Additionally, PRP has shown promise in enhancing oocyte quality, blastocyst formation, and embryo quality in women with decreased ovarian function (Parvanov et al., 2022; Sills et al., 2018). A double-blind randomized controlled study in 2024 also indicated increases in the total number of retrieved oocytes and mature oocytes, while no difference in euploid blastocyst numbers was observed in the PRP treatment group (Barrenetxea et al., 2024). Both studies showed an increase in mature oocyte numbers, reflecting an improvement in oocyte quality to some extent. However, euploid blastocyst numbers were not evaluated in our study. Comparing these findings with the results of the present study, we can observe similar trends. In this study, PRP injections led to increased AMH levels in all three patients, indicating improved ovarian function. Furthermore, all three patients exhibited increased mature oocytes, and two patients showed improved ovum-to-blastocyst rates, reflecting enhanced oocyte quality.

Upon the administration of PRP, its abundance of these growth factors is believed to be the primary contributor to its positive impact on ovarian tissue regeneration and repair (Masuki et al., 2016). The introduction of PRP into the ovarian tissue may effectively regulate cellular activities, including promoting cell proliferation, facilitating cell differentiation, and encouraging angiogenesis. As a result, this stimulation of ovarian tissue regeneration and repair can lead to improvements in ovarian function and oocyte quality, ultimately resulting in increased levels of AMH and higher pregnancy rates for individuals experiencing POI (Sfakianoudis et al., 2020). These growth factors, such as epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF), are known to promote cell proliferation, angiogenesis, differentiation, and repair, which are vital processes for restoring ovarian functionality (Amable et al., 2013). EGF is known to promote cell proliferation and differentiation, which may lead to increased follicular cell growth and enhanced follicular development in the ovaries (Luo et al., 2020). FGF is crucial for ovarian tissue regeneration and repair, as it stimulates ovarian angiogenesis, leading to improved blood supply to the ovaries, thereby, promoting tissue repair and regeneration (Reynolds and Redmer, 1998). VEGF plays a critical role in ovarian tissue regeneration by stimulating the formation of new blood vessels, which enhances oxygen and nutrient supply to the ovarian tissue, facilitating tissue repair and regeneration (Reynolds and Redmer, 1998). PDGF, released by platelets, is essential for cell proliferation and repair processes, contributing to the restoration of ovarian functionality (Bouet et al., 2020).

Our study has limitations. First, the sample size is small, with only three cases analyzed. Large-scale studies with a more diverse patient population are necessary to validate the findings and establish the generalizability of the results. Second, the study lacks a control group, making it challenging to directly compare the outcomes with a non-PRP-treated group. Future research should include randomized controlled trials to provide stronger evidence for the efficacy of PRP in improving ovarian function and oocyte quality in women with ovarian failure. Additionally, the number of euploid blastocysts should be measured to further confirm the improvement in oocyte quality due to PRP.

In conclusion, the results of this study suggest that intraovarian PRP injections hold promise as a potential therapeutic intervention for women with ovarian failure.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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