



Effect of 5% Allogeneic Platelet-Rich Plasma Supplementation on GDF-9 and BMP-15 Secretion During the IVM of GV Oocytes From Women With PCOS: A Pilot Quasi-Experimental Study

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Abstract

Objectives: Polycystic ovary syndrome (PCOS) is associated with insulin resistance and impaired oocyte competence. In vitro maturation (IVM) is a viable alternative, and supplementation with platelet-rich plasma (PRP) may improve outcomes. However, previous studies have primarily focused on autologous PRP. This study aimed to evaluate the effect of 5% allogeneic PRP supplementation in IVM medium on oocyte maturation in women with PCOS, using growth differentiation factor-9 (GDF-9) and bone morphogenetic protein-15 (BMP-15) as surrogate biomolecular markers of oocyte maturation.

Materials and Methods: This quasi-experimental study was conducted between 2024 and 2025. Immature germinal vesicle (GV)-stage oocytes (n=24) from patients with PCOS were allocated to either a non-PRP group (n=12 oocytes) cultured in standard G-IVFTM medium or a PRP group (n=12 oocytes) cultured in G-IVFTM supplemented with 5% allogeneic PRP. After 24 hours, GDF-9 and BMP-15 concentrations in the culture media were quantified using ELISA.

Results: Post-IVM, GDF-9 and BMP-15 levels were significantly higher in the PRP group (1.036 ± 0.004 pg/mL and 0.888 ± 0.008 pg/mL, respectively) than in the non-PRP group (1.032 ± 0.003 pg/mL and 0.878 ± 0.004 pg/mL) (*P*=0.020 and *P*=0.001, respectively). Multivariate analysis confirmed that PRP supplementation was the only significant variable associated with increased GDF-9 (*B*=0.004, *P*=0.047, *R*²=33.7%) and BMP-15 (*B*=0.012, *P*=0.002, *R*²=50.7%) levels. Although the absolute changes were small (Δ GDF-9 = 0.004 pg/mL; Δ BMP-15 = 0.012 pg/mL), large effect sizes were observed (Cohen's *d* = 1.13 and 1.58).

Conclusions: Supplementing IVM medium with 5% allogeneic PRP was associated with increased GDF-9 and BMP-15 levels, which are considered surrogate biomarkers of oocyte maturation.

Keywords: Platelet-rich plasma, Growth differentiation factor 9, Bone morphogenetic protein 15, Polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder and a leading cause of infertility among women of reproductive age. This condition is characterized by hormonal imbalance, chronic anovulation, and low-grade inflammation (1). PCOS is closely associated with insulin resistance, with approximately 50–70% of patients developing hyperinsulinemia secondary to hyperandrogenism (2). Insulin resistance disrupts the hypothalamus-pituitary-ovary axis regulation, triggering the dysregulation of folliculogenesis and oocyte maturation. Consequently, oocyte competence is reduced, and pregnancy outcomes in women with PCOS become suboptimal (3,4).

In vitro maturation (IVM) has emerged as an alternative infertility treatment, particularly for patients with PCOS

and insulin resistance (5). The success of this procedure largely depends on the ability of the culture medium to support the oocyte–cumulus complex interaction (6,7). Various efforts have been made to enhance culture media through supplementation with biological materials, such as platelet-rich plasma (PRP). PRP is a plasma concentrate enriched with bioactive growth factors that regulate follicular and tissue growth (8).

Recent studies have evaluated the use of PRP in IVM medium. Rhee et al. (9) reported that supplementation with 5% autologous PRP improved embryo quality and quantity in patients with a history of poor embryo development. Similarly, Rezaie et al (10) reported that supplementation with 5% autologous PRP significantly increased the proportion of mature oocytes. Although these findings highlight the potential of PRP as a culture

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Key Messages

- ▶ The expression of GDF-9 and BMP-15 is essential for folliculogenesis and oocyte maturation, making them representative biomolecular indicators of oocyte competence.
- ▶ Supplementation with 5% allogeneic PRP was associated with increased GDF-9 and BMP-15 levels.
- ▶ Allogeneic PRP is a promising and practical supplement for improving oocyte competence in infertility cases.

medium supplement, the studies used autologous PRP supplementation, which requires additional blood collection from patients undergoing an IVM cycle. Therefore, allogeneic PRP represents a more practical and efficient alternative. However, research on allogeneic PRP supplementation, particularly in PCOS, has not been extensively explored. This indicates promising potential for further investigation.

The optimal follicular microenvironment for oocyte maturation relies heavily on the dynamic interaction between the oocyte and cumulus cells, mediated by oocyte-secreted factors (OSFs) such as growth differentiation factor-9 (GDF-9) and bone morphogenetic protein-15 (BMP-15). The expression of GDF-9 and BMP-15 is essential for folliculogenesis and oocyte maturation, making them representative biomolecular indicators of oocyte competence.

Objective

This study aimed to evaluate the effects of 5% allogeneic PRP supplementation in IVM medium for women with PCOS. Specifically, we assessed the effect of this supplementation on oocyte competence by measuring the levels (or secretion) of GDF-9 and BMP-15 as key biomolecular indicators of oocyte maturation.

Patients and Methods

Participants

Women undergoing in vitro fertilization (IVF) treatment at the Permata Hati Infertility Clinic, Dr. Sardjito General Hospital, Yogyakarta, with anti-Müllerian hormone (AMH) levels >4.45 were consecutively recruited beginning in January 2024. Eligible participants met the Rotterdam 2003 diagnostic criteria for PCOS and satisfied predefined inclusion and exclusion criteria. Insulin resistance status was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR); participants with HOMA-IR values >2.0 were classified as insulin-resistant and included in the study. Only germinal vesicle (GV)-stage oocytes retrieved during ovum pick-up (OPU) procedures and deemed inappropriate for fertilization were included for further analysis.

Study Design

This quasi-experimental study was conducted between

2024 and 2025. GV-stage immature oocytes were consecutively collected during OPU procedures. Thirteen patients were enrolled in the study, comprising nine in the control group and four in the PRP group. From these participants, 24 GV-stage oocytes were obtained and included in the analysis, with 12 oocytes allocated to the control group and 12 to the PRP-supplemented group.

PRP Preparation

A 5% concentration for PRP supplementation was selected based on findings from preliminary studies indicating the optimal efficacy of this concentration for supporting oocyte maturation. PRP was obtained from a single healthy female donor aged 20–30 years with a history of spontaneous pregnancy and no history of reproductive disorders, to minimize inter-donor variability. Approximately 35 mL of peripheral blood was collected and subjected to a two-step centrifugation process: 2,400 rpm ($103 \times g$) for 10 min (first spin), followed by 3,600 rpm ($230 \times g$) for 15 min (second spin). The supernatant was subsequently aliquoted into 1.5 mL microtubes and stored at -80°C until use. The protocol was conducted according to the routine clinical protocol. However, the details regarding PRP preparation, including the quantitative platelet count, were not recorded.

In Vitro Maturation (IVM) Procedure

Cumulus–oocyte complexes (COCs) from GV-stage immature oocytes were fully denuded using HYASE-10X™ (Vitrolife, Sweden), followed by mechanical stripping with 140–170 μm micropipettes. Denudation was performed to eliminate granulosa cells, thereby making it possible to accurately identify oocytes at the GV stage. Oocytes were cultured for 24 hours at 37°C in either the non-PRP group medium (G-IVF™, Vitrolife, Sweden) or the PRP-supplemented group medium (G-IVF™ + 5% PRP). Oocyte maturation stages were determined by microscopic evaluation based on nuclear and cytoplasmic maturation criteria (smooth cytoplasm, intact zona pellucida, and extrusion of a fine polar body) after 24 hours of culture. Oocytes were classified as mature upon reaching metaphase II (MII).

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was performed using culture medium aspirated after 24 hours of culture. Concentrations of GDF-9 and BMP-15 were measured using commercially available human ELISA kits (Catalogue Nos. EH216RB and EH47RB, Invitrogen, USA). Conditioned media obtained from individual denuded oocytes yielded approximately 10 μL per sample, so samples were diluted 1:50 prior to analysis to ensure sufficient working volume and measurement reproducibility. Absorbance was measured at 450 nm, and concentrations were calculated from standard curves generated for each assay according to the manufacturer's instructions. The protocol was based

on the sandwich ELISA principle, which employs capture antibodies, biotinylated detection antibodies, and a streptavidin-horseradish peroxidase detection system. All reagents and incubation times were followed according to the manufacturer's instructions.

Statistical Analysis

Bivariate analysis, including the independent t-test (parametric) and Mann-Whitney test (non-parametric), was performed to assess the differences between groups. Multivariate analysis was performed using multiple linear regression to assess which variables had an impact on GDF-9 and BMP-15 levels. Statistical significance was set at $P < 0.05$.

Results

Baseline Characteristics

In this study, suspected PCOS cases were identified based on AMH measurement. The baseline characteristics of the participants showed that in the PRP (n=4) and non-PRP groups (n=9), the moderate PCOS phenotype predominated (75% and 44.4%, respectively). The severe phenotype was observed in 25% of the PRP group and 33.3% of the non-PRP group, while the mild phenotype was only found in the non-PRP group (22.2%). This pattern indicated that the phenotype distribution in both groups was relatively comparable, with a predominance of the moderate type, consistent with an intermediate clinical profile within the PCOS spectrum. Analysis of the homogeneity of baseline characteristics showed no significant differences between the PRP and non-PRP groups with respect to age, duration of infertility, body mass index (BMI), HOMA-IR, AMH levels, or PCOS severity ($P > 0.05$), indicating that both groups had comparable baseline characteristics and were thus suitable for comparison (Table 1).

Table 1. Baseline Characteristics of Subject Study

	PRP (n = 4)	Non-PRP (n = 9)	Total (n = 13)	P
Age (year)	35.5 ± 4.51	33.33 ± 2.74	34.00 ± 3.34	0.300 ^a
Duration of infertility (year)	8.63 ± 4.66	6.94 ± 1.77	7.46 ± 2.86	0.350 ^a
BMI (kg/m ²)	23.4 ± 0	23.29 ± 4.3	23.5 ± 3.88	0.981 ^a
HOMA-IR	1.69 ± 0.95	1.47 ± 0.73	1.54 ± 0.77	0.664 ^a
AMH (ng/mL)	8.5 ± 3.95	6.46 ± 2.45	7.09 ± 2.98	0.273 ^a
PCOS				0.798 ^b
Mild	0 (0.0%)	2 (22.2%)	2 (15.4%)	
Moderate	3 (75.0%)	4 (44.4%)	7 (53.8%)	
Severe	1 (25.0%)	3 (33.3%)	4 (30.8%)	
Oosit number	12	12	24	

Notes: ^aIndependent t-test, ^bMann Whitney test.

Table 2. Baseline Characteristics of Oocyte Sample From Subject Study

	PRP (n=12)	Non-PRP (n=12)	Total (n=24)	P
GDF-9 baseline (pg/mL)	1.032 ± 0.003	1.031 ± 0.004	1.031 ± 0.003	0.824 ^a
BMP-15 baseline (pg/mL)	0.879 ± 0.005	0.877 ± 0.003	0.878 ± 0.004	0.100 ^a

Notes: ^aIndependent t-test.

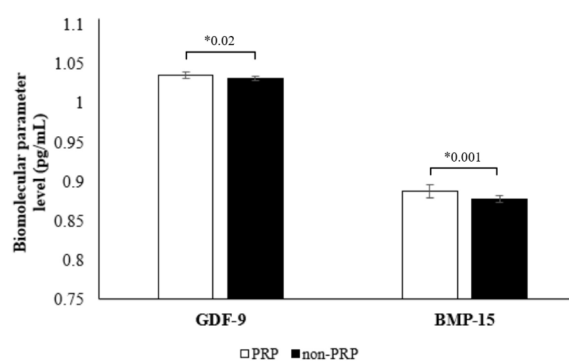


Figure 1. PRP supplementation increases GDF-9 and BMP-15 levels compared with the non-PRP group. * $P < 0.05$ was considered statistically significant (independent t-test)

Prior to the intervention, there were no differences in baseline GDF-9 and BMP-15 levels between the groups, demonstrating homogeneity in the biomolecular profiles of the oocyte samples (Table 2).

Effect of IVM Culture Medium Intervention on Biomolecular Parameters

Independent t-test analysis (Figure 1) showed that two key biomolecular parameters involved in oocyte maturation, GDF-9 and BMP-15, were significantly higher in the PRP group compared to the non-PRP group. The mean post-IVM GDF-9 level was higher in the PRP group (1.036 ± 0.004 pg/mL) than in the non-PRP group (1.032 ± 0.003 pg/mL; $P = 0.020$). Similarly, BMP-15 levels were significantly higher in the PRP group (0.888 ± 0.008 pg/mL) than in the non-PRP group (0.878 ± 0.004 pg/mL; $P = 0.001$). The effect sizes, expressed as Cohen's d, were 1.13 for GDF-9 and 1.58 for BMP-15. These values indicate a large effect of PRP on both biomolecular parameters.

On the other hand, bivariate analysis demonstrated that

GDF-9 and BMP-15 levels were correlated with nuclear maturation ($P=0.046$ and 0.028 , respectively). BMP-15 also correlated with cytoplasmic maturation, particularly with fine polar body formation ($P=0.016$) after PRP supplementation (Table 3).

Multivariate Analysis of Potential Variables on the Status of Oocyte Maturation Based on Biomolecular Parameters
 Multivariate analysis indicated that PRP significantly influenced GDF-9 levels ($P=0.047$). The regression coefficient, $B=0.004$, indicates that PRP supplementation increased GDF-9 levels by 0.004 pg/mL. The coefficient of determination (R^2) was 0.337 , indicating that PRP accounted for 33.7% of the variation in GDF-9 levels (Table 4).

PRP also had a significant effect on BMP-15 levels ($B = 0.012$; $P=0.002$), with a model contribution of 50.7% ($R^2 = 0.507$) (Table 5). Other variables, including age, BMI, duration of infertility, and AMH, did not significantly affect GDF-9 and BMP-15 levels.

Discussion

As a supplementary agent, PRP contains a number of growth factors, including platelet-derived growth factor,

transforming growth factor beta-1 (TGF- β 1), epidermal growth factor (EGF), vascular endothelial growth factor, basic fibroblast growth factor, hepatocyte growth factor, and insulin-like growth factor 1 (11), which are essential at every stage of oocyte maturation. These factors activate various biological pathways that promote cell proliferation and migration, angiogenesis, collagen synthesis, and protection against cellular stress and damage (12). Furthermore, these growth factors appear to be involved in improving oocyte maturation outcomes by elevating key maturation biomarkers such as GDF-9 and BMP-15. This aligns with previous findings that showed a significant increase in GDF-9 and BMP-15 levels.

Multivariate analysis confirmed that PRP supplementation was the variable that significantly increased GDF-9 and BMP-15 levels. These findings indicate that PRP exerts a positive biological influence on the molecular mechanisms involved in oocyte maturation. GDF-9 and BMP-15 are OSFs belonging to the TGF- β superfamily. Both factors play crucial roles in the regulation of oocyte maturation, communication between the oocyte and cumulus cells, and follicular development (13). In PCOS, reduced expression of GDF-9 and BMP-15 has been consistently reported and

Table 3. Correlation of Post-treatment GDF-9 and BMP-15 Levels With Oocyte Maturation After PRP Supplementation

	Nuclear			Smooth cytoplasm			Zona pellucida			Fine polar body		
	Mature	Immature	P	Mature	Immature	P	Mature	Immature	P	Mature	Immature	P
GDF-9	1.04 ± 0	1.032 ± 0.003	0.046*	1.03 ± 0	1.033 ± 0.002	0.276	1.03 ± 0	1.033 ± 0.004	0.837	1.03 ± 0	1.033 ± 0.004	0.321
BMP-15	0.89 ± 0.01	0.879 ± 0.005	0.028*	0.89 ± 0.01	0.88 ± 0.006	0.106	0.88 ± 0.01	0.88 ± 0.005	0.215	0.89 ± 0.01	0.879 ± 0.005	0.016*

Table 4. Multiple Linear Regression Analysis of Factors Associated With GDF-9 Levels

Variable	Model I B (P)	Model II B (P)	Model III B (P)	Model IV B (P)	Model V B (P)
PRP	0.004 (0.018)	0.005 (0.015)	0.005 (0.012)	0.004 (0.045)	0.004 (0.047)
Age (year)		0.0001 (0.393)	0.0001 (0.468)	0.001 (0.238)	0.001 (0.232)
BMI (kg/m ²)			0.0001 (0.306)	0.0001 (0.283)	0.0001 (0.264)
Duration of infertility				0.001 (0.323)	0.001 (0.347)
AMH (ng/mL)					0.0001 (0.689)
R ²	0.228	0.255	0.294	0.332	0.337

Table 4. Multiple Linear Regression Analysis of Factors Associated With BMP-15 Levels

Variable	Model I B (P)	Model II B (P)	Model III B (P)	Model IV B (P)	Model V B (P)
PRP	0.010 (0.001)	0.013 (0.001)	0.013 (0.001)	0.013 (0.001)	0.012 (0.002)
Age (year)		-0.001 (0.100)	-0.001 (0.111)	-0.001 (0.705)	-0.001 (0.937)
BMI (kg/m ²)			0.0001 (0.993)	0.0001 (0.996)	0.0001 (0.887)
Duration of infertility				0.0001 (0.807)	0.001 (0.598)
AMH (ng/mL)					0.0001 (0.622)
R ²	0.427	0.498	0.498	0.500	0.507

is associated with disrupted paracrine communication between the oocyte and cumulus cells, follicular arrest, and reduced oocyte maturation competence (14). In this study, PRP supplementation increased GDF-9 and BMP-15 levels, indicating improved oocyte secretory activity. Biologically, this effect is likely related to the presence of TGF- β family growth factors in PRP, which can activate SMAD signaling pathways, including SMAD2/3 and SMAD1/5/8. Physiological activation of these pathways regulates the expression of genes involved in cumulus cell function, oocyte metabolism, and cytoplasmic quality, thereby supporting enhanced oocyte maturation competence, even under denuded oocyte conditions (15).

At the molecular level, GDF-9 binds to BMPRII and ALK5 receptors expressed on granulosa and cumulus cells, activating the SMAD2/3 pathway (15) (Figure 2). This activation then stimulates the expression of genes involved in cumulus expansion, such as *HAS2*, *PTX3*, and *TNFAIP6*, which contribute to hyaluronic acid matrix formation around the oocyte. This matrix serves as a protective layer while supporting intercellular communication and indirectly contributing to the cytoplasmic maturation of the oocyte (16,17).

Beyond its role in oocyte–cumulus cell communication, GDF-9 supports oocyte maturation by mitochondrial activity enhancement, cytoskeletal reorganization, synthesis of proteins required for spindle formation, and progression to the MII stage (18). Physiologically, GDF-9 secretion by the oocyte is relatively low at the GV stage and increases as maturation progresses toward MII, in line with increased protein synthesis and cytoplasmic maturation. In this study, post-IVM GDF-9 levels in the PRP group were relatively stable and were significantly

higher than those in the non-PRP group. This suggests that PRP helps maintain optimal GDF-9 expression throughout oocyte maturation, even in the absence of paracrine support from cumulus cells (15,18,19).

Physiologically, BMP-15 functions synergistically with GDF-9 to regulate oocyte–cumulus cell communication and support oocyte cytoplasmic maturation (20) (Figure 2). By binding to BMPRII/ALK6 receptors, BMP-15 activates the SMAD1/5/8 pathway, promoting the expression of cumulus expansion genes and supporting increased mitochondrial activity and intracellular redox balance in the oocyte. BMP-15 also interacts with the EGF receptor (EGFR) pathway, thereby strengthening signals involved in the maturation process (15,21,22). GDF-9 and BMP-15 have been reported to act synergistically through formation of an active heterodimer, cumulin, capable of activating both SMAD2/3 and SMAD1/5/8 pathways, which are implicated in granulosa cell proliferation and oocyte developmental competence (23,24). In the present denuded oocyte model, the modest increase in BMP-15 observed in the PRP group may suggest a potential autocrine response to bioactive components present in PRP. However, as downstream signaling pathways were not directly assessed, these mechanisms should be interpreted as biologically plausible explanations rather than confirmed molecular effects. Consequently, PRP supplementation may contribute to a microenvironment supportive of oocyte maturation, although requires further investigation.

Limitations of the Study

The study has several limitations that should be addressed in future research. First, the study included

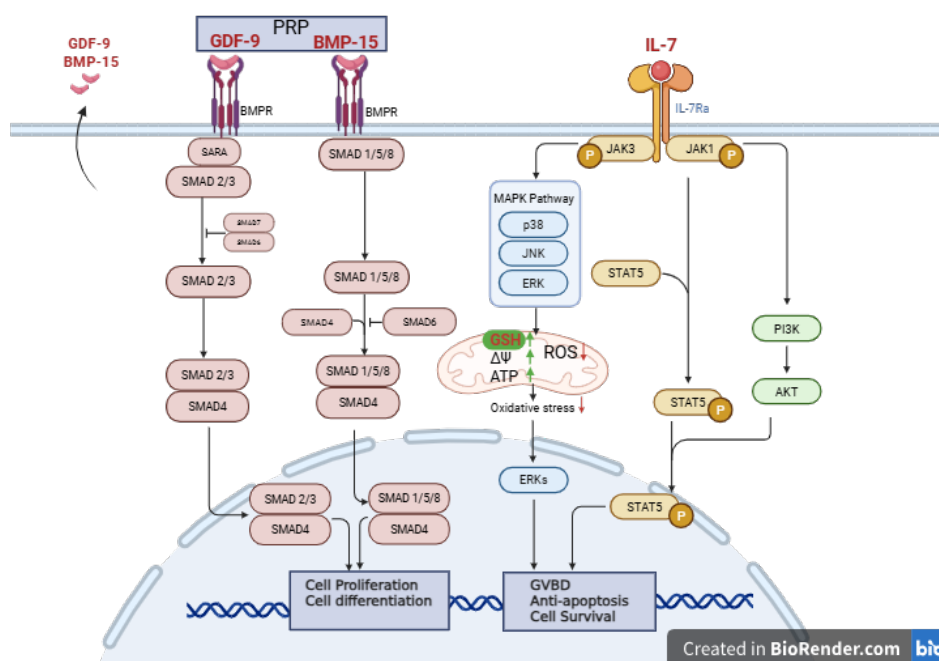


Figure 2. Intracellular signaling pathways involving GDF-9 and BMP-15 in oocyte maturation.

only 13 participants and 24 GV-stage oocytes, which limits statistical power and increases the risk of type II error. Second, the relatively small sample size meant that more than one oocyte was obtained from some participants to achieve the minimum required oocyte number. This may introduce potential clustering bias and limit the generalizability of the findings to a broader population. Third, the quasi-experimental nature of the study, with unequal group sizes and the absence of formal randomization, may introduce selection bias and confounding.

Conclusions

The supplementation with 5% allogeneic PRP was associated with increased GDF-9 and BMP-15 levels, which are considered surrogate biomarkers of oocyte maturation. These findings indicate that allogeneic PRP is a promising and practical supplement for improving oocyte competence in infertility cases. Future studies should include larger patient cohorts with a greater number of oocytes to enable more robust statistical analyses and subgroup evaluations, and should adopt randomized controlled trial (RCT) designs with balanced allocation to strengthen causal inference.

Authors' Contribution

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Supervision: Dewi Kartikawati Paramita, Djaswadi Dasuki, Anto Sawarno.

Validation: Tiara Kusumaningtyas, Dewi Kartikawati Paramita, Djaswadi Dasuki.

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Writing—original draft: Tiara Kusumaningtyas, Annisa Nur Islahi, Intan Kusumaningtyas, Berli Kusuma, Nabila Ramiza Puteri.

Writing—review & editing: Tiara Kusumaningtyas, Annisa Nur Islahi, Intan Kusumaningtyas, Berli Kusuma, Nabila Ramiza Puteri.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

This study was approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta (protocol number: KE/FK/0022/EC/2023). Written informed consent was obtained from all participants prior to enrollment.

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