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*Original Research*

# Aberrant Expression of the Epidermal Growth Factor Family in Women With Different Polycystic Ovary Syndrome Subtypes: An Observational Case-Control Study

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**Abstract**

**Background:** Alterations in epidermal growth factor (EGF) family members in patients with polycystic ovary syndrome (PCOS), a highly heterogeneous condition, remain controversial. This case-control study investigated EGF family levels in PCOS subtypes and analyzed their correlation with *in vitro* fertilization (IVF) pregnancy outcomes. **Methods:** Follicular fluid samples were collected from 94 women with PCOS and 81 control women who underwent IVF embryo transfer and serum samples were obtained from a subset of the same cohort, including 58 women with PCOS and 32 controls. EGF and amphiregulin (AREG) levels were evaluated. EGF and AREG levels in the serum and follicular fluid of patients with PCOS were analyzed by subtype. The correlation between follicular fluid AREG levels and IVF pregnancy rates in PCOS, particularly in subtype A (anovulation [AO] + hyperandrogenism [HA] + polycystic ovaries [PCO]), was investigated. EGF receptor (EGFR) gene expression was measured by real-time PCR. **Results:** Serum EGF levels were significantly elevated, whereas serum AREG levels were lower in patients with PCOS. Changes in serum EGF and AREG levels across PCOS subtypes were consistent with the overall disease phenotype. Follicular fluid AREG levels were significantly lower in PCOS subtype A compared with controls. Pregnancy rates showed a decreasing trend in the PCOS subtype A group, potentially related to the decreased follicular fluid AREG levels in these patients, although validation requires a larger sample size. Real-time PCR analysis revealed higher expression of the EGFR gene in the ovarian follicular granulosa cells from women with PCOS than in controls. **Conclusions:** This study found altered expression of EGF, the EGF family member AREG, and the EGFR gene in patients with PCOS.

**Keywords:** epidermal growth factor; amphiregulin; epidermal growth factor receptor; pregnancy rate; polycystic ovary syndrome

## 1. Introduction

Polycystic ovary syndrome (PCOS) is a reproductive endocrine disorder characterized by chronic anovulation, hyperandrogenism, and polycystic ovaries, with a global prevalence ranging from 5% to 10% [1]. While PCOS is the leading cause of anovulatory infertility in reproductive-age females [2], the molecular basis for the disruption of folliculogenesis in women with PCOS remains incompletely understood [3].

The epidermal growth factor (EGF) family plays a critical role in promoting cell division and differentiation. EGF family members, including EGF and EGF-like factors such as amphiregulin (AREG), epiregulin (EREG), and betacellulin, exhibit highly similar structural and functional characteristics [4]. Recent research has uncovered the key role of the EGF-like network as a mediator of luteinizing hormone (LH) signaling in the induction of ovulation [5], and dysregulation of this network may impair oocyte maturation [6]. Previous studies have reported aberrant expressions of EGF and AREG in the blood or follicular fluid of women with PCOS [7,8], and altered AREG expression in granulosa cells may affect *in vitro* fertilization (IVF) outcomes [9]. Thus, aberrant expression and activity of the EGF family, particularly AREG, may contribute to oocyte developmental incapacity in PCOS, consequently impacting pregnancy outcomes in these patients. However, one study reported that women with PCOS do not exhibit a similar change in clinical pregnancy rates when undergoing IVF [10]. The mechanism underlying this discrepancy and whether the EGF family may potentially influence pregnancy outcomes in women with PCOS after IVF remains unclear.

In this study, considering the heterogeneity of PCOS, we aimed to provide a possible explanation to reconcile

this discrepancy. We hypothesized that EGF family members were aberrantly expressed in different subtypes and could be potentially correlated with IVF outcomes. Thus, we systematically studied the expression of EGF and the EGF family member AREG in serum and follicular fluid in women with PCOS, analyzing expression levels by subtype. We also examined the relationship between alterations in AREG and reduced IVF outcomes in certain patients with PCOS. We further investigated the expression of the EGF receptor (EGFR) in the ovarian follicular granulosa cells of women with PCOS by subtype.

## 2. Materials and Methods

### *2.1 Study Population*

This case-control study was approved by the Ethics Committee of Peking University Third Hospital (2019SZ-048). Written informed consent was obtained from all participants prior to their inclusion in the study. This study included 94 women with PCOS and 81 control patients who had undergone IVF/intracytoplasmic sperm injection-embryo transfer (ICSI-ET) for the first time from Jun 2021 to Oct 2023 in Peking University Third Hospital. The diagnosis of PCOS was made following the Rotterdam consensus criteria, which require the presence of two or more of the following: oligo-ovulation and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, or polycystic ovarian morphology [11]. Following the 2003 Rotterdam criteria, the 94 participants with PCOS were divided into four subtypes on the basis of phenotype: 40 subtype A individuals with chronic anovulation (AO), hyperandrogenism (HA), and polycystic ovaries (PCO) (AO + HA + PCO); 42 subtype B individuals with chronic anovulation and polycystic ovaries without clinical or biochemical hyperandrogenism (AO + PCO); 3 subtype C individuals with chronic anovulation and hyperandrogenism but normal ovarian morphology (AO + HA); and 9 subtype D individuals with hyperandrogenism and polycystic ovaries but regular ovulatory cycles (HA + PCO). Ovarian morphology was evaluated using standardized ultrasound criteria (follicle number per ovary). The control group consisted of 81 age-matched women with infertility because of tubal or male factors and who had regular menstrual cycles without any clinical or biochemical signs of hyperandrogenism or polycystic ovaries. Women who received hormonal therapy within 3 months prior to the study were excluded from participation.

### *2.2 IVF/ICSI-ET Process and Definitions*

Control subjects underwent controlled ovarian stimulation and IVF/ICSI cycles were managed using a long protocol of pituitary down-regulation with gonadotropin-releasing hormone (GnRH) agonist (Triptorelin Acetate, Ipsen Pharma Biotech, France). To ensure consistency and comparability, PCOS patients included in this study were given a GnRH agonist long protocol in this study, either. After controlled ovarian stimulation, recombinant gonadotropin (Gonal F, Merck-Serono, Beijing, China) was administered with doses adjusted according to follicular growth and serum estradiol levels. To ensure AREG expression free from the interference of human chorionic gonadotropin (hCG), Recombinant hCG (Ovidrel, Merck-Serono, Darmstadt, Germany) was administered at the same dose of 250 µg when at least three follicles reached a diameter of 18 mm. Oocytes were collected using transvaginal ultrasound-guided follicular aspiration 34 to 36 h post-hCG administration, after which luteal phase support was provided. Based on sperm quality, either conventional IVF or ICSI was performed. Two embryos were transferred 3 days after oocyte retrieval. The fertilization rate was defined as the number of oocytes exhibiting two pronuclei after insemination divided by the total number of oocytes inseminated. The two pronuclei (2PN) rate was defined as the ratio of the number of 2PN zygotes to the total number of metaphase II oocytes. A positive pregnancy was defined as a plasma β-hCG level greater than 5 IU/L on day 14 post-embryo transfer, and a clinical pregnancy was diagnosed by ultrasonographic visualization of fetal heartbeat.

### *2.3 Sample Collection and Biochemical Assays*

Follicular fluid collection: One milliliter of follicular fluid was aspirated from the leading follicle during oocyte retrieval and centrifuged at 3000 rpm for 10 min to separate erythrocytes and leukocytes. The supernatant was stored at –80 °C for subsequent EGF and AREG assays.

Mural granulosa cell collection: After identifying cumulus-oocyte complexes, the remaining follicular fluid was pipetted into Ficoll gradients and centrifuged at 3000 rpm for 20 min at 4 °C to separate erythrocytes and leukocytes. The fine layer of granulosa cells was collected for RNA extraction.

Serum collection: Serum was obtained from patients on days 2 to 4 of the natural cycle and on the day of hCG trigger to assess basal hormonal conditions and hormonal changes during IVF cycles. Serum samples were collected from 58 women with PCOS and 32 control individuals on the day of oocyte retrieval for the detection of EGF and AREG. The levels of EGF and AREG in serum and follicular fluid were determined using human anti-EGF and anti-amphiregulin enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). Serum follicle-stimulating hormone, LH, testosterone, and estradiol levels were detected using a chemiluminescence immunoassay with the Immulite 2000 (Diagnostic Product Corporation, Los Angeles, CA, USA).

### *2.4 RNA Extraction and Real-Time Polymerase Chain Reactions (PCR)*

Total RNA was extracted from ovarian granulosa cells from 22 participants with PCOS and 28 control individuals using a total RNA kit I (Omega Bio-tek, Nor-

cross, GA, USA). Total RNA was then reverse transcribed and amplified into cDNA using a reverse transcription kit (TIANGEN Biotech, Beijing, China), followed by real-time polymerase chain reactions (PCR) using SYBR Green real-time PCR Master Mix (Toyobo, Osaka, Japan). A DNA Engine Opticon 2 System (Bio-Rad, Hercules, CA, USA) was used for real-time PCR analysis. Gene expressions were normalized to those of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and determined using the $2^{-\Delta\Delta CT}$ method. The primer and probe sequences were as follows: EGFR: forward 5′-TGCGTCTCTTGCCGGAAT-3′, reverse 5′-GGCTCACCCTCCAGAAGGTT-3′; GAPDH: forward 5′-AGAAGGCTGGGGCTCATTTG-3′, reverse 5′-AGGGGCCATCCACAGTCTTC-3′. All primer/probe sets were purchased from Sangon Biotech, Inc. (Shanghai, China).

### *2.5 Statistical Analysis*

All values are presented as mean ± standard error of mean (SEM). A receiver operating characteristic (ROC) curve was constructed to determine the cut-off value of follicular fluid AREG for predicting IVF outcomes. The optimum cut-off values were determined primarily by their sensitivity and specificity. Statistical analysis was performed using either a two-tailed unpaired Student's *t*-test or one-way analysis of variance. SPSS version 16.0 (IBM Corp, Armonk, NY, USA) was used for analyses, and $p < 0.05$ was considered statistically significant.

## 3. Results

### *3.1 Participant Characteristics*

The general characteristics of participants, including age, duration of infertility, pregnancy history, and body mass index, were comparable between the PCOS and control groups (Table 1). Not surprisingly, patients with PCOS had more antral follicles compared with the control group, and individuals with PCOS subtype A had a higher body mass index, higher antral follicle count, lower basal follicle-stimulating hormone levels and higher basal LH levels compared with the control group, with statistical significance ($p < 0.05$) (Table 1).

### *3.2 Aberrant Serum EGF and AREG Levels in PCOS Women and Decreased Follicular Fluid AREG Levels in PCOS Subtype A*

The serum levels of EGF and AREG were evaluated in samples from the PCOS and control groups. ELISA tests showed that serum EGF levels were significantly elevated in women with PCOS ($p < 0.01$) (Fig. 1A). The level of AREG in serum was significantly lower in the PCOS group compared with the control group ($p < 0.01$) (Fig. 1B). In contrast to the aberrant EGF and AREG serum levels in the PCOS group, the EGF and AREG levels in follicular fluid showed no statistical difference between women with PCOS and controls (Fig. 1C,D), despite the fact that AREG levels in the follicular fluid of both groups were thousands of times greater than those in serum (Fig. 1B,D).

Given that PCOS is a disease with high heterogeneity, we investigated whether EGF family levels showed differences in the four PCOS phenotypes and the control group. The alterations in serum EGF levels among PCOS subtype groups were generally consistent with the overall manifestation of PCOS (Fig. 2A). Lower serum AREG levels were only observed in PCOS subtype B (AO + PCO) ($p < 0.01$) (Fig. 2B). No marked difference in follicular fluid EGF levels was observed between the four PCOS phenotypes and the control group (Fig. 2C). The follicular fluid AREG levels in PCOS phenotype A (AO + HA + PCO) were significantly decreased compared with the control ($p < 0.01$), while the levels in the other two subtypes showed no difference compared with the control group (Fig. 2D).

### *3.3 A Trend of Poorer IVF Outcomes in Patients With PCOS Phenotype A*

We next investigated whether IVF parameters and outcomes differed between PCOS phenotype A, the overall PCOS group, and the control group. Regardless of the higher gonadotropin dosage in the control group, and although there was no significant difference in IVF parameters between the PCOS group or PCOS subtype A and the control group (Table 2), we observed a decreasing trend in the pregnancy rate in the PCOS subtype A group (33.3%) compared with the control group (54.7%) ($p = 0.098$) (Fig. 3A), while the IVF pregnancy rates between other three PCOS subtypes (47.1%) and the control group (54.7%) were generally comparable. Moreover, the live birth rates of the PCOS group, PCOS subtype A, and the control group shared a similar trend with the pregnancy rate, closely resembling the mirror image of the follicular fluid AREG levels in PCOS and its subtypes (Figs. 2D,3A).

### *3.4 A Tendency of Reduced IVF Outcomes in Patients With PCOS Subtype A and Lower Follicular Fluid AREG*

To explore whether the trend of poorer IVF outcomes in patients with PCOS subtype A was possibly associated with the aberrant expression of AREG in follicular fluid, we further analyzed the correlation between the levels of follicular fluid AREG and the IVF outcomes. Although not statistically significant, the pregnancy rate of patients with lower follicular fluid AREG than the cut-off values tended to be decreased compared with those with higher follicular fluid AREG levels (data not shown). Among patients with PCOS subtype A, individuals with successful IVF outcome tended to have higher follicular fluid AREG levels compared with those with poor IVF outcome (Fig. 3B). Furthermore, the difference within the PCOS subtype A group exhibited a tendency toward statistical significance (area under the ROC curve [AUC] = 0.703, $p < 0.07$) (Fig. 3C) with the optimum cut-off value 96.61 ng/mL (Sensitiv-

**Table 1. General characteristics of the study participants (mean ± SEM).**

| | Control group (n = 81) | PCOS group (n = 94) | PCOS Subtype A (n = 40) | PCOS Subtype B + C + D (n = 54) |
|---|---|---|---|---|
| Age (years) | 31.21 ± 0.41 | 30.94 ± 0.43 | 30.98 ± 0.55 | 30.91 ± 0.64 |
| Duration of infertility (years) | 4.63 ± 0.32 | 5.46 ± 0.34 | 5.58 ± 0.45 | 5.37 ± 0.50 |
| Pregnant history (times) | 0.69 ± 0.11 | 0.56 ± 0.10 | 0.62 ± 0.13 | 0.53 ± 0.18 |
| Body mass index (kg/m$^2$) | 22.61 ± 0.34 | 23.62 ± 0.41 | 24.29 ± 0.62* | 23.03 ± 0.53 |
| Basal FSH level (IU/L) | 6.70 ± 0.20 | 6.16 ± 0.20 | 5.97 ± 0.30* | 6.29 ± 0.27 |
| Basal LH level (IU/L) | 4.27 ± 0.33 | 7.53 ± 0.53** | 8.90 ± 0.54** | 6.56 ± 0.66** |
| LH/FSH | 0.66 ± 0.47 | 1.08 ± 0.71** | 1.59 ± 0.96** | 1.29 ± 0.86** |
| Antral follicle count (numbers) | 10.76 ± 0.33 | 23.83 ± 0.58** | 25.88 ± 1.03** | 22.91 ± 0.60** |

SEM, standard error of mean; PCOS, polycystic ovary syndrome; FSH, follicle-stimulating hormone; LH, luteinizing hormone; *, Compared with the control group $p < 0.05$; **, Compared with the control group $p < 0.01$.

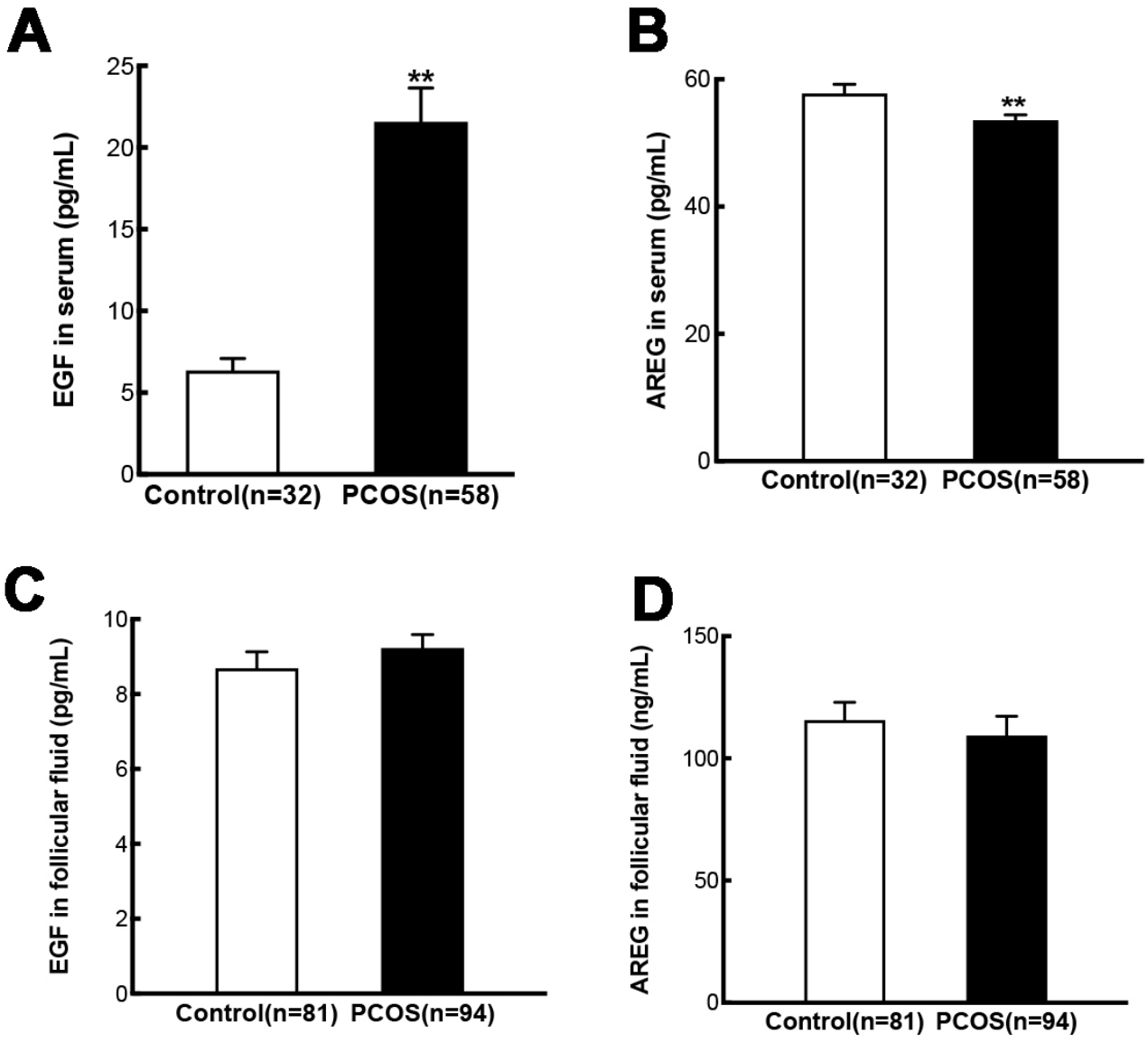


**Fig. 1. Epidermal growth factor (EGF) and amphiregulin (AREG) levels in serum and follicular fluid of women with PCOS**. (A) Serum EGF was elevated in the PCOS group. (B) Serum AREG was lower in the PCOS group. (C) Follicular fluid EGF in the PCOS group was comparable to that of the control group. (D) Follicular fluid AREG displayed no difference between the PCOS and control groups. (**$p < 0.01$).

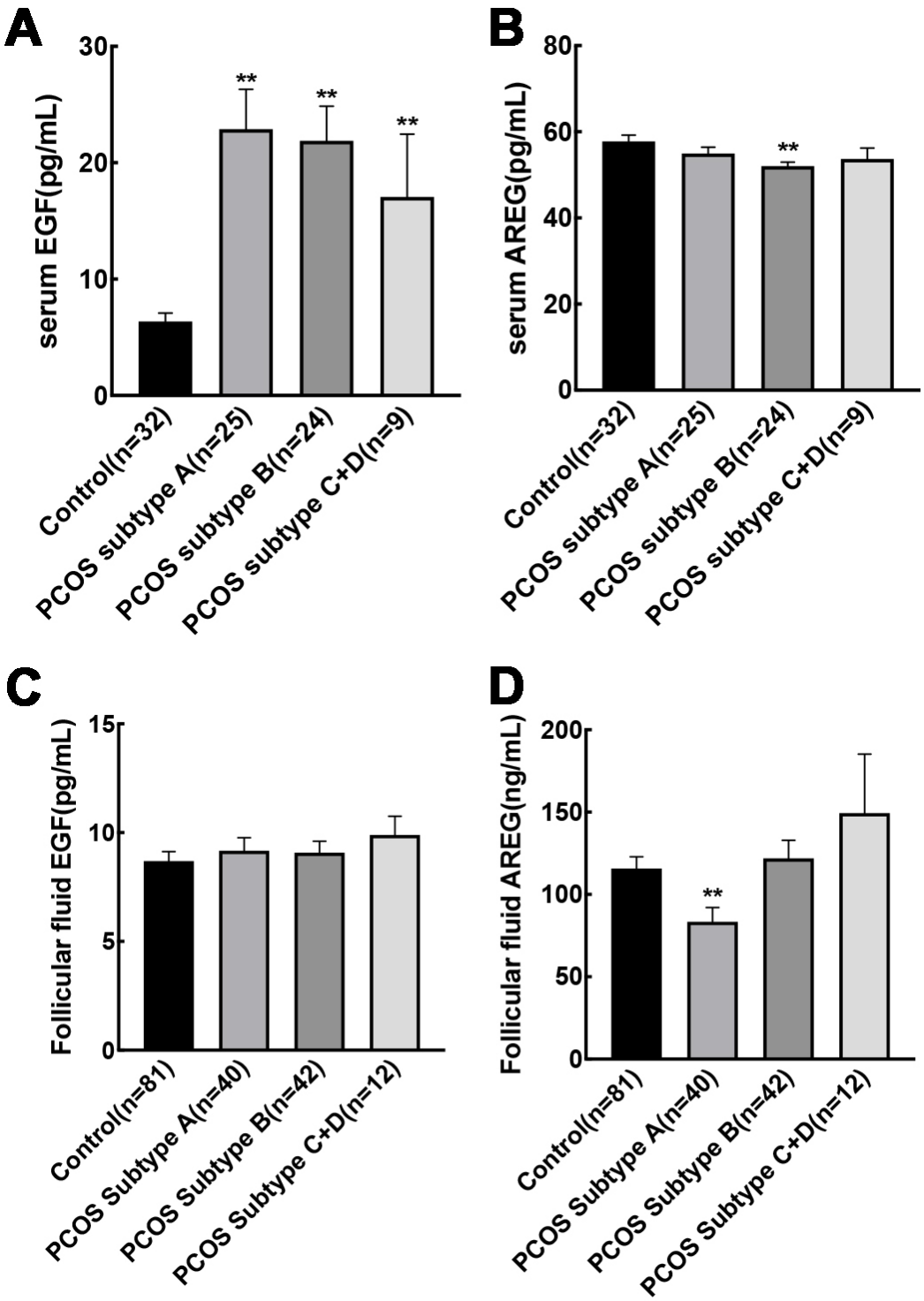


**Fig. 2. EGF and AREG levels in serum and follicular fluid of women with PCOS subtypes**. (A) Elevated serum EGF in different PCOS subtypes was consistent with the overall manifestation of PCOS. (B) Lower serum AREG was observed in PCOS subtype B. (C) Follicular fluid EGF did not show significant differences among different PCOS phenotypes. (D) Follicular fluid AREG was markedly decreased in PCOS subtype A compared with the control group. (**$p < 0.01$).

ity 75.0% and Specificity 68.8%, respectively), indicating a potential association between the lower levels of follicular fluid AREG in patients with PCOS subtype A and the tendency for reduced IVF outcomes. As these data did not reach statistical significance, the correlation between lower follicular fluid AREG and the tendency of reduced IVF out-

**Table 2. *In vitro* fertilization parameters and outcomes of participants (mean ± SEM).**

| | Control (n = 81) | PCOS (n = 94) | PCOS subtype A (n = 40) |
|---|---|---|---|
| Gn dosage (IU) | 2638.11 ± 101.44 | 2049.65 ± 97.64** | 1824.69 ± 136.04** |
| LH on hCG day (IU/L) | 1.36 ± 0.15 | 1.36 ± 0.14 | 1.68 ± 0.27 |
| E2 on hCG day (pmol/L) | 14,542.31 ± 817.29 | 14,726.62 ± 1016.36 | 17,329.82 ± 1800.21 |
| Oocyte retrieval (numbers) | 15.42 ± 6.89 | 16.97 ± 10.02 | 18.05 ± 10.25 |
| Fertilization rate (%) | 66.11 ± 2.39 | 69.43 ± 2.21 | 66.79 ± 3.80 |
| 2PN rate (%) | 56.25 ± 2.74 | 55.95 ± 2.55 | 52.99 ± 3.92 |

hCG, human chorionic gonadotropin; E2, estradiol; 2PN, two pronuclei; **, Compared with the control group $p < 0.01$.

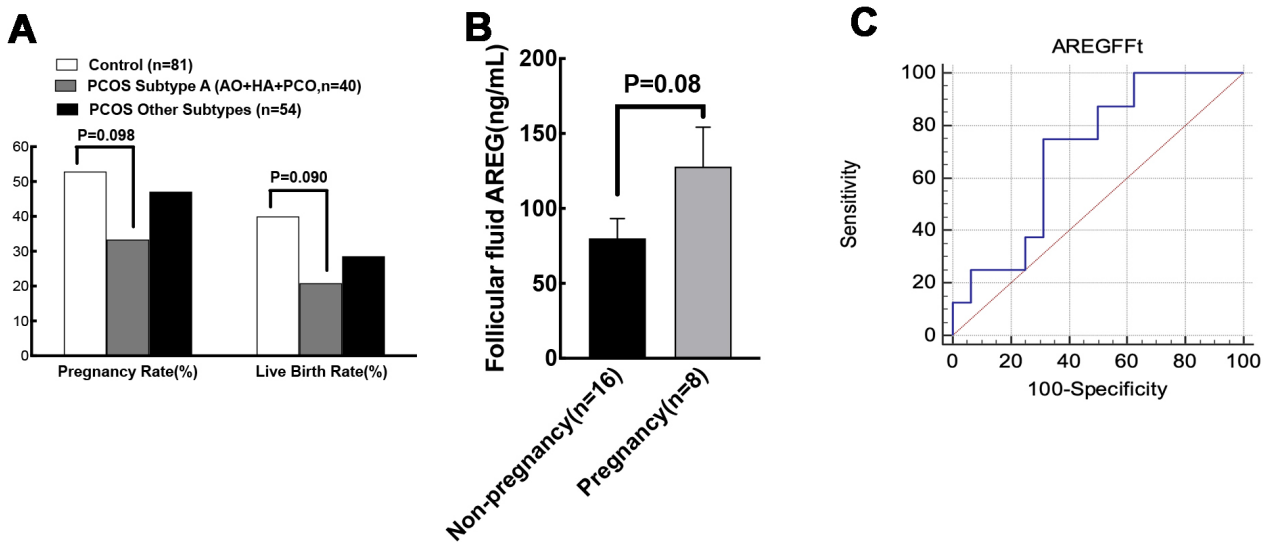


**Fig. 3. A trend of reduced *in vitro* fertilization (IVF) outcomes correlating with lower follicular fluid AREG in patients with PCOS subtype A**. (A) Pregnancy and live birth rates in PCOS subtype A showed a decreasing trend compared with the control group. (B) Among patients with PCOS subtype A, pregnant individuals tended to have higher follicular fluid AREG levels compared with non-pregnant individuals. (C) The receiver operating characteristic (ROC) curve indicated a potential association between decreased follicular fluid AREG levels and lower IVF pregnancy rates, which showed a tendency toward statistical significance.

comes in patients with subtype A still required a larger sample size to be validated.

### *3.5 Upregulation of EGFR Expression on Ovarian Follicular Granulosa Cells in Women With PCOS*

To assess EGFR gene expression in women with PCOS, we performed real-time PCR. The results showed that the expression of the EGFR gene in ovarian follicular granulosa cells was markedly increased in the PCOS group compared with the control group ($p < 0.05$) (Fig. 4A). EGFR gene expression levels were also slightly increased in all PCOS subtypes compared with the control group (Fig. 4B); however, these results did not display statistical significance, likely due to the limited sample sizes of individual subgroups.

## 4. Discussion

The paracrine/endocrine system within the intrafollicular environment plays a crucial role in oocyte maturation [12]. Park et al. [6] discovered that EGF-like growth factors act as mediators of LH action in the ovulatory follicle, and one report showed that oocyte maturation depends on LH-stimulated accumulation of AREG [13]. Considering that the folliculogenesis process is disrupted in women with PCOS, it is unsurprising that EGF family members are aberrantly expressed in these patients and are involved in this process. Previous studies have substantiated this hypothesis. Proteomics analysis indicated that AREG was downregulated in follicular fluid samples from patients with PCOS [8], and serum EGF was also reported to be elevated in women with PCOS [7]. Despite these findings, the pregnancy outcomes of women with PCOS undergoing IVF did not show differences compared with the control group [14]. Given that PCOS is a highly heterogeneous disease, we hy-

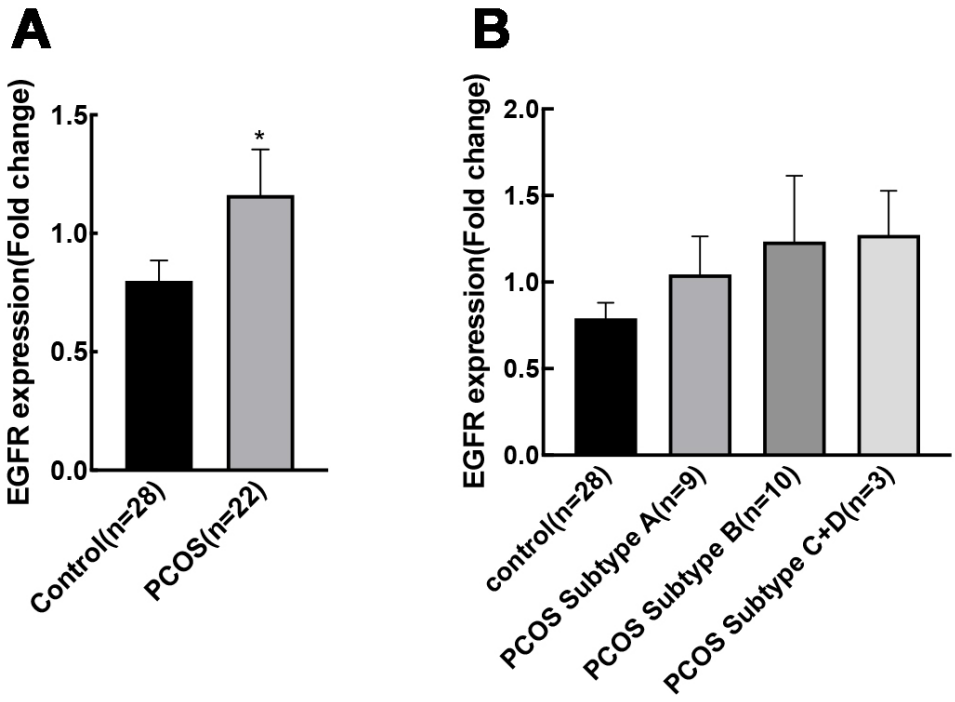


**Fig. 4. EGFR gene expression in women with PCOS**. (A) EGFR gene expression was significantly increased in the PCOS group. (B) EGFR gene expression tended to be elevated in all PCOS subtypes, although without statistical significance. (*$p < 0.05$).

pothesized that the expression of the EGF family may vary among different PCOS subtypes. The results of this study verified our hypothesis. We observed an elevation in EGF and a reduction in AREG levels in serum samples from patients with PCOS. In our subtype analysis, we found that serum AREG levels were significantly lower only in PCOS subtype B (AO + PCO). Notably, our study suggested that individuals with PCOS subtype A (AO + HA + PCO) have a decreased level of follicular fluid AREG. Moreover, the decreased level of follicular fluid AREG in PCOS subtype A was potentially associated with poorer IVF outcomes for this population, providing a possible explanation for the discrepancy between AREG alterations and the overall similar pregnancy outcomes in women with PCOS.

EGF and EGFR signaling pathways regulate fundamental functions, including cell survival and proliferation. Serum EGF has been reported to be elevated in women with PCOS and is an independent risk factor affecting the natural conception rate and pregnancy outcomes in this population [7]. Consistent with previous studies, we found that serum EGF levels were significantly elevated in women with PCOS, while serum EGF levels were comparable among PCOS subtypes. Nevertheless, we did not observe any alterations in follicular fluid EGF levels in the PCOS group. As EGF inhibits LH receptor expression and interacts with testosterone in regulating cell development [15,16], it is reasonable to speculate that a higher serum EGF level may be correlated with elevated LH levels and hyperandrogenism in women with PCOS, thus contributing to poorer pregnancy outcomes. However, whether EGF levels can predict IVF outcomes remains unclear. Given that there was no difference in follicular fluid EGF levels between women with PCOS and the control group, and that serum EGF levels were indistinguishable among PCOS subtypes, we did not consider EGF a candidate marker for predicting IVF outcomes in the PCOS population. A previous study has reported that AREG in follicular fluid is a thousandfold higher than in serum and is much more abundantly expressed than EGF in follicular fluid [17]. In this study, we additionally found a divergent change trend of EGF and AREG in serum and follicular fluid. Considering EGF expression could be influenced by multiple factors, while AREG is mainly synthesized by granulosa cells and functions in the follicle microenvironment, we focused our research interest on AREG in follicular fluid.

AREG is essential for mediating gonadotropin-dependent stimulation of oocyte maturation and emerges dramatically under hCG stimulation [18]. AREG expression was reported to increase in a dose- and time-dependent manner after hCG or LH stimulation [18,19]. In this study, all patients received the same dose of hCG (250 µg), and all follicular fluid samples were collected at the same time post-hCG injection (34–36 h), ensuring that the AREG alterations in PCOS conformed to reality. In contrast to a previous quantitative proteomic study reporting reduced AREG expression in the follicular fluid of patients with PCOS [8], we did not observe a statistical difference in follicular fluid AREG levels between the PCOS and control

groups, although patients with PCOS subtype A exhibited significantly lower AREG levels. This discrepancy may be attributed to the different proportions of PCOS subtype A participants in the two studies. Theoretically, the diminished AREG in PCOS subtype A could impair cumulus-oocyte complex matrix expansion, leading to poorer pregnancy outcomes [20,21]. Because of the limited sample size of this study, we constructed a ROC curve instead of performing multiple logistic regression analysis to evaluate the correlation between follicular fluid AREG and the IVF pregnancy rate. While there was no strong correlation between follicular fluid AREG and IVF pregnancy rates in the overall study population (data not shown), we found that they were potentially correlated in PCOS subtype A. Moreover, non-pregnant women with PCOS subtype A who underwent IVF tended to have decreased follicular fluid AREG levels, indicating a possible predictive effect of AREG on IVF outcomes in patients with PCOS subtype A. The validation of the specific relationship between follicular fluid AREG levels and IVF outcomes, however, required multiple linear regression analyses in a larger-scale study. And the potential underlying mechanism deserves to be addressed in subsequent research.

PCOS subtype A, characterized by chronic anovulation, hyperandrogenism, and polycystic ovaries (AO + HA + PCO), is the most common and severe subtype of PCOS [22,23]. According to a previous report, PCOS subtype A has over 44% prevalence in European countries and the USA [24,25]. Our previous studies found no differences in IVF pregnancy rates between women with and without PCOS [10,14]. Our current study provided similar results. However, the metabolic, hormonal, and reproductive traits of patients with PCOS can vary significantly among different PCOS subtypes [26,27]. Few studies have focused on the IVF outcomes of patients with PCOS by subtype. In this study, we discovered a tendency toward a decreased IVF pregnancy rate in PCOS subtype A. Because of the limited sample size of this study, we could not exclude the possibility of bias based on the baseline information of the patients. Thus, a larger-scale study is needed to verify this finding.

EGFR has been reported to be overexpressed in women with PCOS, as assessed by immunofluorescence [28,29]. Consistent with previous studies, we found that EGFR mRNA was significantly overexpressed in the ovarian follicular granulosa cells of patients with PCOS, indicating its upregulation at the transcriptional level in the internal environment of PCOS. We did not find significant aberrant expression of EGFR in certain PCOS subtypes. All EGF family members exert their effects by binding to EGFR. Therefore, further studies are needed to elucidate how the aberrant EGF (family)-EGFR signaling pathway participates in the pathophysiologic changes of PCOS.

*Limitations*

Our study has several limitations. First, the relatively small sample size of our study inevitably introduced greater statistical bias and impacted statistical power, thus limiting the generalizability of the findings. A case in point is EGFR gene expression, which was significantly increased in the PCOS group while no significant differences were observed among PCOS subtypes. It is entirely possible that EGFR gene expression in some subgroups (Subtype C and D, etc.) was underestimated due to the small sample size. Second, because of the limited sample size, we did not conduct multiple logistic regression analysis when assessing the correlation between follicular fluid AREG and IVF pregnancy rates. This limitation could potentially undermine the robustness of the results. Third, we assessed EGF family members and EGFR in patients undergoing IVF cycles. It is likely that expressions may be different in patients with PCOS who are in natural ovulation cycles rather than in a state of superovulation.

## 5. Conclusions

Our exploration of serum and follicular fluid EGF levels and the EGF family member AREG in women with PCOS revealed their aberrant expression. To the best of our knowledge, this study represents the first analysis of EGF family expression in patients with PCOS by subtype and takes the first attempt to investigate its correlation with IVF outcomes. We observed a tendency for a reduced pregnancy rate in the PCOS subtype A group (AO + HA + PCO), which correlated with the follicular fluid AREG levels in these patients. Additionally, we found that EGFR was upregulated at the transcriptional level in women with PCOS.

## Availability of Data and Materials

All data reported in this paper will also be shared by the lead contact upon request.

## Author Contributions

NL and ML contributed to the conception and design, experimental studies, data acquisition, and manuscript preparation. RL contributed to data interpretation and manuscript review. YC and JQ contributed to the conception and design, data interpretation, statistical analysis, manuscript preparation, editing, and review, and took responsibility for the integrity of the article as a whole from inception to publication. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

This study was carried out in accordance with the guidelines of the Declaration of Helsinki and approved by

the Ethics Committee of Peking University Third Hospital (2019SZ-048). Written informed consent was obtained from all participants prior to their inclusion in the study.


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## Conflicts of Interest

The authors declare no conflicts of interest. Rong Li is serving as one of the Editors-in-Chief of this journal. We declare that Rong Li had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Michael H. Dahan.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG47657.

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