Comparing GDF9 in mature follicles and clinical outcome in different PCOS Phenotypes

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Research Article

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Abstract

Background:
Polycystic ovary syndrome (PCOS) is the leading cause of anovulatory infertility. Growth differentiation factor 9 (GDF9) is a prime candidate as potential biomarker for the assessment of oocyte competence. Herein, we aimed to screen GDF9 of mature follicles in women with different PCOS phenotypes undergoing controlled ovarian hyperstimulation (COS) and analyse the correlation between GDF9 expression levels and the oocyte developmental ability.

Methods
In this study, follicular fluid (FF) and cumulus cells (CCs) of mature follicles were collected from different PCOS phenotypes. Enzyme linked immunosorbent assay (ELISA) was used to examine the level of GDF9 in FF; Immunohistochemical method was performed to detect GDF9 protein expression in CCs. The independent effect of GDF9 on blastocyst formation and clinical pregnancy was determined by Binary Logistic Regression analysis. Results: The GDF9 levels in FF for phenotype A and B were significantly increased, compared to the phenotype D, (P = 0.019, P = 0.0015, respectively). Increased GDF9 expression in CCs of phenotype A and B was accompanied by the changes of FF. The analysis of the multivariable logistic regression showed that GDF9 was a significant independent prognosticator of blastocyst formation (P = 0.001). The phenotype A had a higher percent of blastocyst formation than the phenotype B and D (P = 0.001).

Conclusions
Taken together, GDF9 expression varied in different PCOS phenotypes. The phenotype A had a higher GDF9 level and even more ability of blastocyst formation.

Background
Polycystic ovary syndrome (PCOS) affects 5%-20% of women in their reproductive age[1] and is considered the most common endocrine and metabolic disorder characterized by oligo-anovulation (OA), hyperandrogenism (HA), polycystic ovarian morphology (PCOM) (≥ 12 follicles per ovary, about 2 ± 9 mm in diameter, and/or augmented ovarian volume > 10 ml), hirsutism, insulin resistance, obesity, and menstrual irregularity[2]. Therefore, PCOS is multifactorial and heterogeneous with variable phenotypes infertility. Diagnosis of different PCOS phenotypes (A, B, C, D) was made according to the Rotterdam criteria in 2003[3]. Diagnosis of different PCOS phenotypes (A, B, C, D) was made according to the Rotterdam criteria. Phenotype A has all the diagnostic features of the syndrome (chronic anovulation, hyperandrogenism and polycystic ovaries on ultrasound). Phenotype B has chronic anovulation and hyperandrogenism, but no polycystic ovaries on ultrasound. Phenotype C includes women who have regular menses but with hyperandrogenism and polycystic ovaries; finally, phenotype D includes women who have irregular menses and polycystic ovaries with ultrasound, but without evidence of hyperandrogenism.

The influence of chronic HA in PCOS negatively affects the physiological androgen wane that occurs when follicular growth progresses. Ovulation induction is often used to treat anovulatory patients with PCOS, but many of these women fail to conceive and resort to assisted reproductive technologies (ART). However, it is also suspected that these PCOS oocytes may be of poor quality as a result of intra- and extra-ovarian factors[4]; this might lead to a lower
fertilization rate, poor embryo quality, a lower implantation rate and a higher miscarriage rate [5–7]. Indeed, it is well known that oocyte competence influences embryonic development. Stefano Palomba's research [8] demonstrates that the capacity of oocyte with PCOS contributes differently to reproductive potential. Researchers believe that it depends largely on the PCOS phenotype and the clinical manifestations associated with PCOS. Ramezanali and colleagues reported phenotypes A and B are considered to the most severe, metabolic disorder [9]. Furthermore, phenotypes C and D represent the mild forms of classic PCOS and may have a different pathogenic pathway. Androgen levels are the major distinguishing endocrine feature differentiating phenotypic expressions of PCOS [10]. To date, only a few studies have assessed the potential impact of different PCOS phenotypes on the outcomes of ART. The causative role of oocyte competence in women with PCOS remains controversial and no clear evidence is available regarding the impact of PCOS and PCOS phenotypes on oocyte competence.

Numerous studies have showed biomolecules with important functions in oocyte development and altered expression in PCOS [4]. And biological molecules variations may be reflected in the FF composition, affecting the microenvironment of oocyte growth [11]. The concentration of PCOS biomolecules in FF provides information about potential biomarkers of oocyte competence. In addition, perturbed fluid physiology has a synergistic effect on abnormal follicular genesis and disordered oogenesis in PCOS [12]. GDF9 is known to be an oocyte-specific paracrine factor. Both oocytes and CCs express GDF9, which is exchanged through gap junctions [13–14]. Previous studies have indicated that higher GDF9 levels in the FF are significantly associated with oocyte maturation and embryo quality [15–16], suggesting a potential relationship between GDF9 levels and oocyte competence. GDF9 is an important biomarker for predicting oocyte development potential. However, the exact relationship between the presence of GDF9 in the follicular development microenvironment and oocyte capacity with different PCOS phenotypes is unclear. The FF and CCs are by-products of in-vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) which can reflect the ovarian microenvironment to a certain extent and directly reflect the oocyte metabolism and quality [17].

Based on these issues, this study aims to discuss the relationship between human oocyte capacity and PCOS. It would be interesting to know the reproductive potential of oocytes from women with different types of PCOS to determine whether oocyte abnormalities contribute to PCOS-related hypofertility. In this study, we detected the expression levels of GDF9 in FF and CCs in dominant follicle. The aim of this present study was to determine whether GDF9 expression varies with different PCOS phenotypes and to analyze the correlation between GDF9 levels and oocyte developmental potential.

**Materials And Methods**

**Study population and sampling**

110 infertile couples who underwent IVF/ICSI between December 2019 and September 2020 at University Hospital were included in this study. The study protocol was approved by the second affiliate hospital of Fujian Medicine University. Review Board and all participants freely signed the informed consent upon enrollment in the study. Polycystic ovary syndrome was diagnosed according to the Rotterdam criteria (Rotterdam ESHRE/ASRM Sponsored PCOS Consensus Workshop Group 2004) and so fulfilled at least two of the following three criteria: oligo- and/or anovulation, hyper-androgenism and polycystic ovary. All of the non-PCOS patients had normal ovarian morphology and regular menstrual cycles with female tubal pathology infertility or male infertility. PCOS patients were categorized as: phenotype A, B, C and D. All patients in our study sought treatment due to irregular menstruation, which resulted in a lack of patients with PCOS phenotype C. Women with a history of pelvic or ovarian surgery, severe endometriosis, anovulation and aneuploidy or a specific disease by preimplantation genetic screening were excluded from the
analysis. Additional exclusion criteria were the use of surgically retrieved spermatozoa, the presence of congenital adrenal hyperplasia, and androgen secreting tumours of Cushing syndrome.

Ovarian Stimulation

The patients were submitted to an individualized COS protocol for IVF procedures chosen according to a the clinical profile, including cause of infertility, age, follicle-stimulating hormone (FSH) levels and antral follicle count (AFC), mainly using follicular phase long-acting gonadotropin-releasing hormone (GnRH) agonist protocol and GnRH antagonist protocol. After ovarian stimulation with gonadotropin-releasing hormone agonist (Serono, Geneva, Switzerland) and recombinant FSH (Serono, Geneva, Switzerland), Three or more follicles reached 17 mm diameter, and then 6000–10000 IU of human chorionic gonadotropin (hCG, Lizhu Inc., Zhuhai, China) was then administered to trigger final maturation. Oocytes were retrieved from under transvaginal ultrasonography-guided follicular aspiration was performed approximately 36 hours after the hCG injection.

Human Ff And Oocyte-cumulus Complex Collection

FF was obtained from the first aspirated follicle which contains a single CCs and collected. FF samples were chilled on ice and then centrifuged at 4°C for 10 min at 300g; One ml clear supernatant was transferred to a microfuge tube and stored at -80°C for subsequent GDF9 assessment. Each oocyte retrieved from the FF of individual follicles had part of its CC mechanically removed with 16-gauge microdissection needles[18]. The CCs were prepared to cell slides.

Measurement Of Gdf9 In Human Ff

FF was diluted 1:5 in phosphate-buffered saline (PBS) and then measured using a human GDF9 ELISA Kit (Elabscience, Wuhan, China) in according to the manufacture instructions. Absorbance was read in an automatic microplate reader at 450 nm. The concentration FF was calculated using a standard curve.

Immunochemistry Staining

Distribution of GDF9 in the CCs was detected by immunohistochemistry staining based on the manufacturer’s instruction (BOSTER Biological Technology Co. Ltd, Wuhan, China). Briefly, the CCscell slices were fixed with 4% paraformaldehyde in phosphate buffer saline for 15 min and immersed in 3% hydrogen peroxide to block endogenous peroxidase activity, and then they were blocked in goat serum for 1h. Slices were incubated with primary antibodies rabbit anti-GDF9(GDF9, Abcam, USA, 1: 100) overnight at 4°C and then in biotin-labeled anti-rabbit secondary antibody for half an hour. Finally, slides were incubated with the peroxidase substrate DAB at room temperature until the desired stain intensity was achieved, lightly counterstained with hematoxylin, and covered with glass cover slips. The signals were examined and photographed by microscope (Leica MZ16FA, Germany). Quantification of immunoreactivity was performed using Image J 6.0, and 3–5 fields were randomly selected from each slide to determine the mean optical density (MOD).

Embryo Quality Assessment And Reproductive Outcome

The embryo morphology assessment was evaluated on days 3, 5, 6, after oocyte retrieval. Blastocysts were scored according to the Gardner grading system[19] and recorded on the base of the expansion stage, inner cell mass and
trophectoderm. Embryo vitrification was performed via a Cyrotop carrier system combined with DMSO-EG-S as cryoprotectants. Embryo thawing was operated in a sequential manner when cyrotop was transferred into dilution solution. The clinical pregnancy was supported by the observation of a gestational sac on ultrasound scanning 4–5 weeks after embryo transfer. And clinical pregnancy rate was calculated on a per transfer cycle.

**Statistical analysis**

The Statistical Package for Social Sciences (SPSS 22.0) and Graphpad Prism version 5 was used for statistical analysis. Quantitative variables were expressed as the mean ± standard deviation (for normally distributed datasets) or as the median [interquartile range (IQR)]. Comparisons between two groups were performed with the One-way ANOVA statistical analysis for parametric conditions and the Mann-Whitney U Test for nonparametric conditions. Comparison of proportion was evaluated by Chi-square Test between groups. To determine the independent effect of GDF9 on blastocyst formation and clinical pregnancy, a Binary Logistic Regression analysis was used after adjustment for well-established, pre-specified confounding factors including BMI, serum HA, AFC, dosage of Gn used, PCOS phenotypes. In the design, the dependent variable is dichotomous (blastocyst formation and clinical pregnancy), and the independent variables are dichotomous variables (serum HA), continuous variables (BMI, AFC, dosage of Gn used,) and ordered multicategorical variables (PCOS phenotypes). In categorical variables (PCOS phenotypes), We designed individually PCOS phenotype A, B, D and analyse the risk of blastocyst formation and clinical pregnancy compared to control groups. All tests were two-tailed, and the threshold for statistical significance was set to *p* < 0.05.

**Results**

**Participant characteristics**

Table 1 shows the clinical and endocrine charactereristics of the phenotype groups. Overall, 71 PCOS patients and 39 control individuals were included. Of these, 29 out of 71 (40.8%) patients had PCOS phenotype A, 18 out of 71 (25.6%) had phenotype B, 24 out of 71 (33.8%) had phenotype D. Of note, There were significant differences between the three PCOS phenotypes and control group in BMI and higher for PCOS phenotype B. (P < 0.001). Serum HA levels were significantly higher for phenotypes A and B than for phenotype D. Phenotypes A and D patients had higher AFC than phenotypes B. According to the ovarian stimulation cycle characteristics, the total dosage of Gn used was significantly higher for phenotype B than for phenotypes A and D. However, the number of follicles ≥ 14mm, number of oocytes retrieved for phenotypes A and D was significantly higher than for phenotype B(*p* < 0.001); and phenotypes A and D were associated with a statistically significantly greater blastocyst formation and clinical pregnancy than phenotypes B(*p* < 0.001); There are no statistically differences between the groups in Age, E2 on hCG injection day, type of fertilization, Ovarian stimulation protocol.
### Table 1
Characteristics and clinical outcome of patients according to PCOS phenotypes [x±s, M(P_{25}, P_{75})].

<table>
<thead>
<tr>
<th>Item</th>
<th>Control Group</th>
<th>PCOS A</th>
<th>PCOS B</th>
<th>PCOS D</th>
<th>F/\chi^2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO. of cycles</td>
<td>39</td>
<td>29</td>
<td>18</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>31.38±4.28</td>
<td>29.83±2.73</td>
<td>29.83±3.91</td>
<td>30.29±3.20</td>
<td>1.328</td>
<td>0.269</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>22.87±2.63</td>
<td>23.53±3.67</td>
<td>27.19±3.07</td>
<td>22.10±2.85</td>
<td>10.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AFC</td>
<td>15 (9,20)</td>
<td>29 (24,31)^a</td>
<td>17 (15,18)^b</td>
<td>27 (24.25,34.5)^c,d</td>
<td>68.502</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum T (ng/ml)</td>
<td>0.31 (0.27,0.34)</td>
<td>0.66</td>
<td>0.82 (0.72,0.93)^e</td>
<td>0.31 (0.21,0.38)^c,f</td>
<td>78.179</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dosage of Gn used (IU)</td>
<td>2486.54±137.63</td>
<td>2317.24±159.60</td>
<td>3175.00±202.5</td>
<td>1949.48±175.46^c</td>
<td>7.228</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E2 on hCG injection day (u mol/L)</td>
<td>4531.51±490.25</td>
<td>6592.79±568.53</td>
<td>4331.17±721.63</td>
<td>5838.38±624.95</td>
<td>3.388</td>
<td>0.021</td>
</tr>
<tr>
<td>NO. of follicles ≥ 14mm</td>
<td>10.54±4.24</td>
<td>14.69±4.63^a</td>
<td>12.17±4.32</td>
<td>15.37±6.29^c</td>
<td>6.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO. of oocytes retrieved</td>
<td>15.54±6.00</td>
<td>21.14±4.20^a</td>
<td>14.22±5.05^b</td>
<td>18.38±7.66</td>
<td>6.989</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blastocyst formation rate(%)</td>
<td>46.6 (232/498)</td>
<td>60.8 (318/523)^a</td>
<td>44.4 (87/196)^g</td>
<td>55.5 (207/373)^c,f</td>
<td>20.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical pregnancy rate(%)</td>
<td>56.9(33/58)</td>
<td>70.7(29/41)^a</td>
<td>48.3(14/29)^g</td>
<td>64.7(22/34)</td>
<td>4.176</td>
<td>0.243</td>
</tr>
<tr>
<td>Type of fertilization(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.056</td>
<td>0.798</td>
</tr>
<tr>
<td>IVF</td>
<td>79.5(31/39)</td>
<td>82.8(24/29)</td>
<td>72.2(13/18)</td>
<td>75.0(18/24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICSI</td>
<td>20.5(8/39)</td>
<td>17.2(5/29)</td>
<td>27.8(5/18)</td>
<td>25.0(6/24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovarian stimulation protocol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.559</td>
<td>0.479</td>
</tr>
</tbody>
</table>

BMI: body mass index; T: testosterone; AFC: antral follicle count; AMH: anti-Müllerian hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E_2: estrogen; PRL: prolactin; Gn: gonadotropin; F: the Kruskal Wallis H Test for nonparametric conditions: two-more independent samples; \chi^2: Chi-square Test for comparison of proportion; ^a P<0.01, compared with Control Group; ^b P<0.01, compared with PCOS A; ^c P<0.01, compared with PCOS B; ^d P<0.01, compared with Control Group; ^e P<0.01, compared with Control Group; ^f P<0.01, compared with PCOS A; ^g P<0.01, compared with PCOS A;
<table>
<thead>
<tr>
<th>Item</th>
<th>Control Group</th>
<th>PCOS A</th>
<th>PCOS B</th>
<th>PCOS D</th>
<th>F/χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular phase long-acting GnRH agonist protocol</td>
<td>66.7(26/29)</td>
<td>82.8(24/29)</td>
<td>72.2(13/18)</td>
<td>79.2(19/24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GnRH antagonist protocol</td>
<td>33.3(13/39)</td>
<td>17.2(5/29)</td>
<td>27.8(5/18)</td>
<td>20.8(5/24)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index; T: testosterone; AFC: antral follicle count; AMH: anti-Müllerian hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E₂: estrogen; PRL: prolactin; Gn: gonadotropin; F: the Kruskal Wallis H Test for nonparametric conditions: two-more independent samples; χ²: Chi-square Test for comparison of proportion; aP<0.01, compared with Control Group; bP<0.01, compared with PCOS A; cP<0.01, compared with PCOS B; dP<0.01, compared with Control Group; eP<0.01, compared with Control Group; fP<0.01, compared with PCOS A; gP<0.01, compared with PCOS A;

**Gdf9 In The Ff Of Pcos Patients**

Figure 1 summarizes GDF9 levels in the FF of all participants, and the expression of GDF9 in the three PCOS phenotypes and control group was compared. The median GDF9 levels in FF was 7.35ng/ml, (interquartile range 4.47-13.49ng/ml). We found that the level of GDF9 in phenotype D was markedly lower compared with those in control group. No statistically significant differences were observed between the other groups and control group.

**Gdf9 Expression In Ccs**

Likewise, the roles of GDF9 in follicles were also explored by CCs. Because oocytes were usable to culture embryo and transfer, the corresponding CCs was remained to analyse the expression of GDF9. Immunohistochemical staining of CCs of the three PCOS phenotypes and control group is shown in Fig. 2. GDF9-positive cells were detected in CCs and stained with brown cytoplasm. The phenotype D had less GDF9-positive location in CCs compared to phenotypes A and B. The staining intensity for GDF9 in phenotype A (0.2592±0.01505) and phenotype B (0.2407±0.02748) were higher than control group (0.1388±0.008261). The difference was statistically significant (P < 0.05). Interestingly, GDF9 expression in phenotype D (0.1566±0.007416) was similar to control group. This suggests that the expression of GDF9 in different PCOS phenotypes is complex, diverse and may be influenced by other factors, such as BMI and testosterone level.

**Analysis Of Multiple Factors Affecting Blastocyst Formation And Clinical Pregnancy In The Three Pcos Phenotypes**

The risk factors associated with blastocyst formation and pregnancy outcomes were explored by logistic regression analysis (Table 2). Blastocyst formation rate was chosen as the dependent factor. And BMI, serum HA, AFC, Dosage of Gn used, GDF9 (categorical variable) and PCOS phenotypes were chosen as independent factors. We found that: (1) GDF9 is a significant independent prognosticator of blastocyst formation, while it had no significant predictive value for the clinical pregnancy when it adjusted for BMI, serum HA, AFC, dosage of Gn used, PCOS phenotypes; (2) The serum HA had a markedly negative influence on the blastocyst formation (OR = 0.321, 95%CI: 0.232–0.443); (3) The phenotype A group had a 3.347 times higher odds of blastocyst formation compared to control group.
Table 2
Analysis of multiple factors affecting blastocyst formation and clinical pregnancy rate in the phenotypes of PCOS

<table>
<thead>
<tr>
<th></th>
<th>blastocyst formation</th>
<th>clinical pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>P</td>
</tr>
<tr>
<td>GDF9 (ng/ml)</td>
<td>0.991 (0.986–0.995)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.936 (0.922–0.950)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum T (ng/ml)</td>
<td>0.321 (0.232–0.443)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AFC</td>
<td>0.995 (0.993–0.997)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dosage of Gn used (IU)</td>
<td>1.000 (1.000–1.000)</td>
<td>0.261</td>
</tr>
<tr>
<td>PCOS phenotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Phenotype A</td>
<td>3.347 (2.862–3.914)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Phenotype B</td>
<td>1.507 (1.214–1.870)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Phenotype D</td>
<td>1.475 (1.318–1.650)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

OR: odds ratio; CI: confidence interval;

Discussion

The specific features of PCOS is OA, HA, and PCOM. These cardinal features, alone or combined, vary in incidence and severity across PCOS phenotypes which is then classified as phenotype A, B, C or D, increasing not only PCOS severity [20] but also the reproductive potential of women with PCOS[21]. In our present study, we evaluated intraovarian GDF9 levels change of the PCOS phenotypes who received IVF treatment. Over the past 10–15 years it has became increasingly clear that GDF9 is a pivotal regulator of folliculogenesis, and increases important bi-directional communication between the oocyte and somatic cells by transzonal projections(TZPs)[22–23]. We observed that there were differences in GDF9 expression in oocytes among each phenotypes. The median GDF9 level in FF was 7.35 ng/ml that was suitable to embryo development. However, the FF GDF9 levels in phenotype D was markedly lower than other phenotypes. Moreover, Consistent with FF, CCs expressed positive staining for GDF9. In this study, the phenotype A and B had more GDF9-positive staining in CCs compared to the phenotype D. We demonstrated that GDF9 was appeared in human FF accompanied by expression in human CCs according to previous reports. It is clear that the bidirectional communication between oocytes and CCs is in favour of balance and development of normal follicular[24]. We found that compared with the phenotype D, both the FF and histological inspection GDF9 protein levels in phenotype A and B increased. GDF9 was involved in regulation of CCs genes expression, a broad range of CCs functions, glycolysis and amino acid uptaked in CCs and transported to the oocyte[25]. We speculated that oocyte quality and embryo quality was different for the various PCOS phenotypes.

In combination with the observation for clinical characteristics and treatment outcome of patients according to PCOS phenotypes, phenotypes A and D were associated with a statistically significantly greater blastocyst formation and clinical pregnancy than phenotypes B. Initial studies considered women with PCOM as an intermediate group between women with and those without PCOS with regard to metabolic dysfunction that displayed similar or better outcomes in IVF cycles compared with the normo-ovulatory population[26]. Although the sufficient amount of GDF9 promoted the
granulosa cells to produce receptor effect on FSH and E2 in favour of the formation of blastocysts [27]. Phenotypes A and D seemed to have the similar formation of blastocysts and clinical pregnancy.

However, to adjuct blastocyst formation and clinical pregnancy of PCOS phenotypes, we designed and analyse the risk of specified confounding factors including BMI, serum HA, AFC, and the used dosage of Gn. In this study, PCOS phenotypes with HA had more GDF9 compared with normo-androgenic counterparts. Based on literature, the androgens are essential in early folliculogenesis and the pre-ovulatory follicular stages [28]. Meanwhile, GDF9 play an important role in the process of follicular development from the recruitment of the primordial follicle to ovulation and even in corpus luteum formation [29–31]. They might have a synergistic effect on folliculogenesis and embryo development by coordinating fluid physiology, specifically in subgroups of obese women with HA [32]. Likewise, the impact of HA on oocyte quality is subject to debate. Indeed, androgens are involved in folliculogenesis, and a hyperandrogenic environment leads to abnormal folliculogenesis, prematurely activated follicles, mitochondrial abnormalities, and failure of meiosis progression to MII. Furthermore, HA is known to induce premature luteinization of the granulosa cells, which prevents them from progressing to physiological atresia. Recent reports considered phenotype A and B were associated with a greater risk of adverse outcomes in pregnancy [33] and PCOS phenotypes with HA were associated with a lower cumulative live birth rates, when compared with normo-androgenic counterparts. Then, there has been increasing evidence regarding of raised BMI and HA on IVF outcomes which may be related to the pathogenesis of PCOS [34–36]. Dyslipidemia plays a potential role in the failure to fertility through inducing oxidative stress [37]. Previous research even mentioned [38] that the modest increase in serum FSH levels by recombinant FSH administration during COS is inversely correlated to the decrease in serum AMH levels that precedes the emergence of a dominant follicle. This hypothesis is reinforced by our finding that for phenotype with PCOM during IVF, the number of follicles $\geq$ 14mm and oocytes retrieved were obviously increased. Spontaneously, this is because once CCs have received enough FSH for a sufficient time, the imbalance between FSH and AMH effects on the control of aromatase expression is corrected, leading to clearing the excess AMH and increased content of E2 within the CCs.

Indeed, the decreased blastocyst formation has been confirmed in phenotype D after logistic regressions to control data for all potential confounders in this study. Our results also suggest that the phenotype A (considered to be the most severe phenotype) is not associated with especially poor reproductive outcomes but a beneficial contribution of oocyte competence to the final results had the most blastocyst formation after adjusting the results for BMI, serum HA, AFC, the used dosage of Gn. This data showed the important function of GDF9 in oocyte development. For PCOS with different phenotypes, GDF9 may be the most suitable biomarker for phenotype A. It seemed that the blastocyst formation and clinical pregnancy didn't increase, accompanied by the rising GDF9 expression. When it adjusted for BMI, serum HA, AFC, the used dosage of Gn, PCOS phenotypes, GDF9 is a significant independent prognosticator of blastocyst formation, while it had no significant predictive value for the clinical pregnancy; And the phenotype A group had a 3.347 times higher odds of blastocyst formation compared to control group. We also verified that HA had a marked lynegative effects on blastocyst formation in our analysis results and phenotype B had the least blastocyst formation.

**Conclusions**

To date, various morphologic parameters have been used to evaluate the oocyte quality and predict the embryo development. The evidence presented in our study support the role of GDF9 produced by oocytes and CCs as a prophetic biomarker of oocyte competence and blastocyst formation for different PCOS phenotypes, especially phenotype A. Our study might prospectively examine the effect of GDF9 on embryo development and clinical outcomes in different PCOS phenotypes when incorporation of clinically relevant characteristics such as BMI, serum HA, AFC, the used dosage of Gn.
However, there are still limitations and deficiencies in this study. We are aware that the limitation in our study is the relatively small participants number because of the strict inclusion criteria applied. Nevertheless, the study sample is highly homogenous and almost all confound factors that could lead to bias are eliminated therefore. All patients in our study lacks patients with PCOS phenotype C, so further studies are warranted to confirm our founding.

**Declarations**

**Authors’ contributions**

Jingjing Cai performed assisted reproductive technology; Jinxiang Wu designed the study; Xiangmin Luo and Jingjing Cai analyzed the data and wrote the paper. Zhengyao Wang and Donghong Huang prepared the original draft. Zixuan Chen collected samples and clinical data of the GDF9 expression; Hui Cao prepared the figure 1 and 2. Jing Chen prepared the table 1 and 2. All authors read and reviewed the final manuscript.

**Conflict of interest**

No potential conflict of interest relevant to this article was reports.

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**Availability of data and materials**

The data that support the study are available upon reasonable request to the corresponding author.

**Ethics approval and consent to participate**

The study was approved by the Ethical Committee of the Second Affiliated Hospital of Fujian Medical University and written informed consent was obtained from all patients.(Reference: 2019-222).

**Competing interests**

The authors declare that they have no competing interests.

**References**


Figures
Figure 1

Distribution of GDF9 in FF from the three PCOS phenotypes and control group. Data are presented as mean±SD. 
\(^d\) \(P<0.01\), compared with Control Group; \(^a\) \(P<0.01\), compared with Control Group; \(^b\) \(P<0.05\), compared with PCOS A; 
\(^c\) \(P<0.05\), compared with PCOS B
Figure 2

Expression of GDF9 in the CCs. A, Immunohistochemical staining of GDF9 expressed in the control group, phenotype A, phenotype B and phenotype D. (Original magnification: ×400, bars, 50 μm). B, Mean density of GDF9 in CCs of the three PCOS phenotypes and control group, a\(P<0.01\), compared with Control Group; b\(P<0.01\), compared with PCOS D; c\(P<0.01\), compared with Control Group; d\(P<0.01\), compared with PCOS D