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Impact of FSHR -29 (G>A) Polymorphism on Ovarian Response and Pregnancy Outcomes in PCOD Patients Undergoing ART

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ABSTRACT

Background: Polycystic ovary disease (PCOD) is a leading cause of anovulatory infertility, often requiring assisted reproductive technology (ART) for successful conception. Genetic polymorphisms, particularly in the follicle-stimulating hormone receptor (FSHR) gene, may influence ovarian response and reproductive outcomes. This study investigates the association of FSHR-29 (rs1394205) polymorphism with ovarian stimulation parameters, gonadotropin sensitivity, and pregnancy outcomes in PCOD patients undergoing ART.

Methods: A retrospective cohort study was conducted at Krishna IVF Clinic, Visakhapatnam, analyzing 224 PCOD patients undergoing ART between July 2015 and December 2020. Participants were stratified into three genotypic groups: GG (n=107, 47.77%), GA (n=85,37.95%), and AA (n=32, 14.29%). Ovarian response, hormone administration levels, embryo quality, clinical pregnancy rates, and live birth rates were assessed. Statistical analysis included ANOVA, Kruskal-Wallis, and logistic regression models, with GG as the reference genotype.

Results: AA genotype carriers exhibited higher gonadotropin requirements for ovarian stimulation (p=0.074), suggesting reduced FSH receptor expression. Despite comparable antral follicle count (AFC) and oocyte yield across genotypes, clinical pregnancy rates were highest in GG (49.53%), followed by GA (47.06%) and AA (37.50%). AA carriers also demonstrated the lowest live birth rates (21.8%) and highest rates of negative pregnancy outcomes (62.5%). However, statistical significance was not established for pregnancy or live birth outcomes.

Conclusion: FSHR-29 polymorphism may influence ovarian response and reproductive success in PCOD patients undergoing ART. While AA genotype carriers required higher FSH doses, exhibited lower pregnancy rates, and had poorer live birth outcomes, the findings warrant further investigation with larger cohorts to validate clinical significance.

Keywords: *FSHR-29 polymorphism, PCOD, ovarian response, assisted reproductive technology, in vitro fertilization, genetic markers, reproductive success.*

1. INTRODUCTION

Infertility affects approximately one in six couples globally, with the World Health Organization (WHO) estimating that this proportion will continue to rise due to factors such as delayed family planning and the growing availability of fertility treatments, particularly in fast-developing regions like Asia (WHO, 2000). As assisted reproductive technologies (ART), including in vitro fertilization (IVF), become more widespread, understanding the molecular mechanisms underlying fertility is becoming increasingly important.

One critical factor in female and male reproduction is the follicle-stimulating hormone (FSH), which regulates the development of reproductive cells. FSH acts through its receptor, FSHR, located on the surface of ovarian granulosa cells, a key site for follicular development and estrogen production (Camp TA *et al.,* 1991; McNeilly AS *et al.,* 1991; Nordhoff V *et al.,* 2011). FSHR is a member of the G protein-coupled receptor (GPCR) family, which primarily activates the Gas/cAMP/protein kinase A signaling pathway, but can also interact with other pathways, such as those involving APPL1 and inositol 1,4,5-triphosphate (IP3), impacting various cellular functions (Means AR *et al.,* 1974; Thomas RM *et al.,* 2011).

The application of genetic markers has become a focal point in reproductive medicine, as advancements in genomic research have opened new avenues for personalizing fertility treatment. While hormonal and functional biomarkers have been traditionally used to assess ovarian reserve and responsiveness, the role of genetic markers—specifically single nucleotide polymorphisms (SNPs)—in predicting treatment outcomes is a topic of active investigation. SNPs have shown potential in forecasting individual responses to pharmacological treatments, although their use has been hindered by inconsistencies observed between genetic and hormonal parameters in some studies (Alviggi *et al.,* 2012; GA. R *et al.,* 2021).

One area of particular interest is the FSHR gene, where several mutations and SNPs have been associated with infertility, highlighting the critical role of FSHR function in reproductive success. Inactive mutations in the FSHR gene have been linked to impaired fertility, with a clear correlation between the loss of FSHR function and infertility traits (Simoni M *et al.,* 1997; Themmen AP *et al.,* 2000). Additionally, SNPs in the FSHR gene have been found to influence ovarian response in women undergoing IVF treatment (Mayorga MP *et al.,* 2000; Simoni M *et al.,* 2002; Morón FJ *et al.,* 2010).

Of particular interest is a polymorphism located at position -29 in the core promoter region of the FSHR gene. Research has demonstrated that the AA genotype at this locus is associated with a reduced ovarian response to stimulation during IVF treatments (Achrekar SK *et al.,* 2009). This polymorphism also correlates with decreased serum estradiol levels in women with hypertension. Functional assays using Chinese hamster ovary (CHO) cells have shown that the A allele at this position results in lower transcriptional activity compared to the G allele, likely due to the disruption of a binding site for the transcription factor cETS-1 (Nakayama T *et al.,* 2006; Wunsch A *et al.,* 2005).

In addition to its role in IVF outcomes, the FSHR -29 polymorphism has been studied in relation to polycystic ovary syndrome (PCOS), a common cause of anovulatory infertility. PCOS is characterized by hyperandrogenism and insulin resistance, with hyperinsulinemia playing a key role in ovarian dysfunction. Increased insulin levels enhance granulosa cell sensitivity to FSH, promoting the growth of follicular cysts and dysregulating steroidogenesis (Govind *et al*,1999, Catteau-Jonard *et al*,2008). Genetic variations in FSHR may contribute to the pathophysiology of PCOS, influencing both ovarian function and response to hormonal stimulation.

This study aims to explore the role of the FSHR-29 polymorphism in the ovarian response to stimulation in women undergoing IVF, with a particular focus on its impact on treatment outcomes and potential associations with PCOS.

2. MATERIAL AND METHODS:

2.1 Study Design

A retrospective cohort study was carried out in a single centre (Krishna IVF Clinic, Visakhapatnam). The cohort was designed and data from patients (n=224) PCOD subjects who underwent ART treatment from July 2015 to December 2020 was extracted from hospital database software (File Maker version 20).

Women were eligible for the data analysis if they had PCOD diagnosed based on Rotterdam's criteria and were attending the clinic for ART treatment. Exclusion criteria included female factors other than PCOD i.e Endometriosis, Adenomyosis, fibroiduterus, women with oocyte donation cycles, those undergoing stimulation protocols other than the long protocol, women with a single ovary, and those with medical complications such as PID. Hence, these women were excluded from the data analysis. Well-informed consent was obtained from all participants before the study, which was conducted in accordance with the Declaration of Helsinki. The study was approved by the Local Institutional Ethics Committee for research on human volunteers, Krishna IVF Clinic, Visakhapatnam, India.

Subjects were stratified into three groups based on FSHR-29 genotypes: GG (n=107, 47.77%, reference group), GA (n=85, 37.95%), and AA (n=32 14.29%).

Table1: -29 promoter region polymorphism of the FSHR gene

-29 GG	Reference Allele/genotype
-29 GA	Heterozygous
-29 AA	Alternate Allele/ genotype

Parameters assessed included antral follicle count (AFC), oocyte yield, hormonal dosage (FSH, LH), Estradiol levels, fertilization rates, embryo quality, clinical pregnancy rates, and live birth rates. Statistical analyses included ANOVA, Kruskal-Wallis, and logistic regression models to evaluate genotype-phenotype correlations, with GG as the reference.

2.2 Stimulation protocol

For ovarian stimulation, long luteal phase GnRH agonist protocol was used. Ovarian suppression was performed using a decapeptyl depot injection containing Triptorelin 3.75mg (Ferring Pharmaceuticals, Saint-Prex, Switzerland), administered intramuscularly between days 18–24 of the menstrual cycle. After 14 days, Suppression efficacy was validated, indicated by serum estradiol levels below 50 pg/ml and an endometrial thickness less than 5mm. Subsequent ovarian stimulation for follicular growth was initiated using daily subcutaneous injections of recombinant human FSH (r-hFSH), with dosages ranging from 150 to 300 IU, tailored according to the patient's age, BMI, and basal follicle count. This regimen was consistently applied throughout the stimulation phase.

Additionally, LH support was tailored based on the individual's LHCGR gene polymorphism. Specifically, patients categorized under the A/A polymorphism did not receive LH support, whereas those in the A/G and G/G groups were administered additional LH at dosages of 37.5 IU and 75 IU, respectively. This personalized LH supplementation was commenced from the first day of ovarian stimulation and was maintained until the end of the cycle, in parallel with the standard r-hFSH stimulation protocol.

2.3 Genotyping

For genetic assessment of polymorphism of the FSHR gene,-29G/A (rs1394205), peripheral venous blood was collected in EDTA vacutainer tubes (Becton Dickinson and Company, Franklin Lakes NJ, USA) and immediately processed for nucleic acid extraction. Genomic DNA was isolated from leukocytes using a modified 'salting out' method as previously described [Nasiri *et al.*, 2005].

Exon-1 of the FSHR gene was amplified by polymerase chain reaction with 1.0 µl (10 Pmol per µl) primers (forward primer: 5'GGTTCTATTTGCTGTGTGCCTTA3', reverse primer: 5'CGGTCAAGGGGCAGAAATATT3') and a 2x master mix containing Taq DNA (Ampliqon, Denmark). Primers were designed using Primer Express software (Life Technologies, Carlsbad, CA, USA).

The amplification was performed with an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 20s, annealing at 60°C for 20s and 72°C for 20s followed by a final extension at 72°C for 5 min. The amplified product was observed on 2% agarose gel electrophoresis and then purified using exonuclease I and shrimp alkaline phosphatase (ExoSAP-IT) enzyme (Affymetrix, Santa Clara, CA, USA). The purified product was sequenced using a big dye cycle sequencing kit on a 3500 Genetic Analyzer (Thermo Fisher Scientific, USA) and evaluated using SeqScape software (Life Technologies).

FSHR-29 polymorphisms and their variants: -29 GG (reference allele/genotype), -29 GA (heterozygous genotype), and -29 AA (alternate allele/genotype).

2.4 Hormone estimation

Blood samples were collected for serum analysis ofEstradiol(E2) and Luteinizing Hormone (LH) post 2 weeks of downregulation, E2 measurements on day 4 and before hcg trigger and serum hCG post 2 weeks of embryo transfer. All measurements were carried by electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany) at NABL accredited clinical chemistry laboratory (Vijaya medical center, Visakhapatnam). The sensitivities of the assays for E2 were 5 pg/mL, 0.10 mIU/mL for LH and <0.1 mIU/mL for hCG. The coefficients of variances for E2 were 2.74% at 189pg/mL and 2.93% at 367 pg/mL, for LH 4.68% at 20mIU/mL and 3.94% at 59.3mIU/mL, for hcG 10.1% at 4.31mIU/mL and 5.49% at 164mIU/mL

2.5 Measured outcomes

Age, marital life (years), and body mass index (BMI) were assessed at the time of treatment. Patients were considered as obese if they had a BMI ≥30. Mean r-hLH dose, mean r-hFSH dose per day and total dosage, and various embryological parameters; the total number of oocytes with maturity status, the rate of fertilization, the number of embryos (grade 1 and grade 2), clinical pregnancy rate, live birth rate and miscarriage rate. The grading of embryos was carried out as per the Veeck's criteria [Veeck *et al.*, 1991].

2.6 Statistical Analysis

Descriptive statistics (mean ± SD, percentages) were calculated for the cohort and stratified by FSHR -29 genotypes (GG, GA, AA). ANOVA or Kruskal-Wallis tests were used for continuous variables, while chi-squared tests assessed categorical outcomes (clinical pregnancy, live birth rates). Regression models for age, BMI, AFC, and marital duration to evaluate associations between FSHR_29 genotypes, ovarian response, hormonal levels, and clinical outcomes. The GG genotype served as the reference category. Logistic regression analyzed clinical pregnancy and live birth rates. A P-value <0.05 was

considered statistically significant. All analyses and Odds ratio plots were performed using were performed using RStudio, 2024.04.2 Build 764,© 2009-2024 Posit Software, PBC.

3. RESULTS

This study evaluated the influence of FSHR-29 polymorphism on key reproductive parameters in polycystic ovary disease (PCOD) subjects undergoing assisted reproductive technology (ART) cycles.

Table2: Association of FSHR -29 Variants with Fertility Parameters, Oocyte Maturation, and Pregnancy Outcomes in IVF Subjects with PCOD (n=224)

Parameter	GG(n=107,47.77%)	GA (85, 37.95%)	AA (32, 14.29%)	P_Value
Antral Follicle count (AFC)	17.35±3.7	16.87±3.82	17.41±3.17	0.26
Marital life, years	5.52±3.12	5.85±3.62	6.13±3.18	0.49
Female BMI (kg/m2)	26.96±4.12	26.62±3.85	27.25±5.16	0.73
Female_Age	28.83±3.33	30.27±3.86	28.32±3.64	0.02
Days of stimulation	10.11±1.33	10.09±1.28	10.41±1.1	0.28
r-hFSH dosage, IU/day	197.86±50.71	197.35±49.87	219.31±58	0.14
Total r-hFSH dose, IU	1987.85±558.48	1985.88±567.54	2285.94±702.73	0.07
Total r-hLH dosage, IU	463.56±266.71	513.12±264.83	473.22±312.08	0.53
Estradiol (E2) (pg/ml) (before hcg trigger)	2112.97±850.89	2193.19±906.89	2094.62±899.04	0.76
Number of Oocytes (n)	18.09±3.03	17.87±3.6	18.41±3.68	0.96
MII (n)	14.59±4.28	14.62±4.34	15±3.17	0.10
MI (n)	1.51±1.83	1.62±1.93	1.41±1.46	0.80
P1 (n)	1.91±2.39	1.4±1.85	1.62±1.7	0.17
Fertlization (%)	84.83±14.76	85.68±15.03	86.59±14.24	0.84
No of embryos formed (n)	6.4±2.61	6.35±2.85	6.72±2.9	0.72
Embryos Transferred (n)	3.17±0.79	3.38±0.72	3.28±0.92	0.23
No of Blastocyst (n)	0.68±0.99	0.74±1.09	0.97±1.2	0.53
Embryo quality (Grade-1) (n)	1.58±1.3	1.62±1.38	1.72±1.4	0.88
Embryo quality (Grade-2) (n)	0.94±1.48	1.09±1.53	0.69±1.23	0.40
Embryos vitrified (n)	3.25±2.89	2.96±3.03	3.44±3.12	0.69
hCG (mIU/mL)a	545.81±828.13	458.66±784.19	212.46±342.89	0.10
Clinical Pregnancy (n, %)	53, 49.53%	40, 47.06%	12, 37.50%	0.48
Live Birth Rate (n, %)	39, 36.45%	28, 32.94%	7, 21.88%	0.66

Data presented are number (percentages) or mean values ± SD, as appropriate for the variable, Shapiro-Wilk tests performed for normality. For normally distributed variables: Used ANOVA (parametric test).

For non-normally distributed variables: Use Kruskal-Wallis test used (non-parametric alternative to ANOVA)

For Categorical variable performed Chi-Square test.

Distribution of Subjects by FSHR-29 Genotype and Baseline Characteristics: Among the 224 PCOD patients undergoing ART, the distribution of FSHR-29 genotypes was as follows: GG (107, 47.77%), GA (85, 37.95%), and AA (32, 14.29%). The mean age of participants varied significantly across groups (p = 0.02), with GA individuals being the oldest (30.3 ± 3.86 years), followed by GG (28.83 ± 3.33 years) and AA (28.32 ± 3.64 years).

Body Mass Index (BMI) was comparable across genotypes, with GG individuals having a mean BMI of 26.96 ± 4.12, GA at 26.62 ± 3.85, and AA at 27.25 ± 5.16 (p = 0.73). The mean Marital life was slightly higher in AA individuals (6.18 ± 3.18 years) compared to GA (5.85 ± 3.62 years) and GG (5.52 ± 3.12 years), (p = 0.49).

The duration of ovarian stimulation was similar across genotypes, with GG and GA individuals requiring approximately 10.11 ± 1.33 and 10.09 ± 1.28 days, respectively, while AA individuals had a slightly longer duration of 10.41 ± 1.1 days (p = 0.28). These findings suggest that while genotype-based differences exist in age and infertility duration, stimulation protocols were largely consistent across groups.

The assessed parameters included antral follicle count (AFC), total follicle-stimulating hormone (FSH) dose, oocyte yield, embryo quality, clinical pregnancy rates, and delivery outcomes. The GG genotype served as the reference, with comparisons made to GA and AA genotypes. The observed trends suggest that FSHR-29 polymorphism may influence ovarian response, gonadotropin sensitivity, and pregnancy outcomes, particularly in AA individuals.

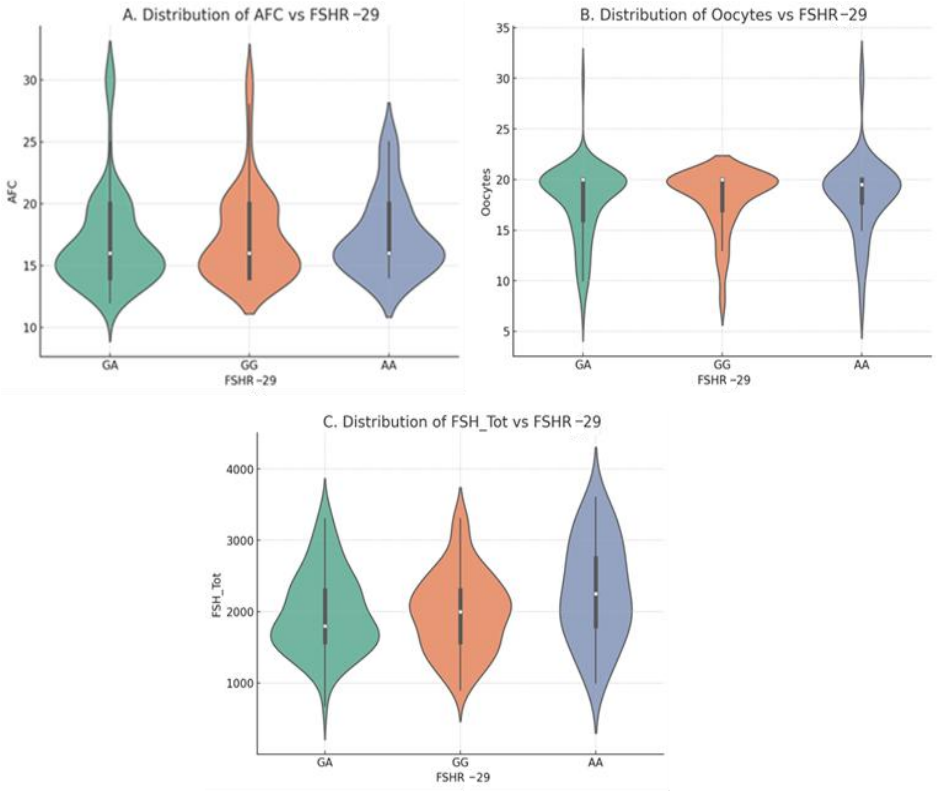

Fig 1.Distribution of Key Ovarian Response Parameters Across FSHR -29 Genotypes in PCOD Patients Undergoing ART. (A) AFC shows slight variation across genotypes. (B) Oocyte yield remains similar across groups. (C) Total FSH requirement trends higher in AA individuals, suggesting reduced ovarian sensitivity, reflecting differences in gonadotropin requirements. Violin plots illustrate data distribution, median, and interquartile range.

The median AFC was identical (16) across GG, GA, and AA genotypes, indicating that FSHR-29 polymorphism does not have a strong influence on ovarian reserve. However, a subtle trend toward lower mean AFC in GA individuals (16.87 ± 3.82) compared to GG (17.35 ± 3.7) and AA (17.41 ± 3.17) was observed, suggesting that GA carriers may have a slightly reduced ovarian reserve (p = 0.26).

A more notable trend was observed in the total r-hFSH doserequired for ovarian stimulation, where AA individuals exhibited the highest gonadotropin requirement (2285.94 ± 702.73 IU) compared to GG (1987.85 ± 558.48 IU) and GA (1985.88 ± 567.54 IU). This suggests that AA carriers may exhibit lower ovarian sensitivity to exogenous FSH, necessitating a higher gonadotropin dose for follicular development. Previous studies have linked FSHR-29 AA polymorphism with reduced receptor expression, which could explain this higher FSH requirement. Although this trend was observed (p = 0.07).

The number of oocytes retrieved across genotypes was comparable, with the median number remaining at 20 for all groups,

suggesting that FSHR-29 polymorphism does not drastically influence total oocyte yield. However, a subtle variation in mean oocyte yield was observed, with AA individuals having a slightly higher yield (18.41) compared to GG (18.09) and GA (17.87) in PCO patients. Nevertheless, this difference was minor and not statistically significant (p = 0.96). Other fertility parameters, including MII oocytes, MI, PI and fertilization rates, showed no significant differences between genotypes (p > 0.05).

Embryo quality analysis revealed differential distributions among genotypes, where GA individuals showed a higher proportion of Grade II embryos, while AA individuals exhibited a slightly higher proportion of Grade I embryos. However, no definitive pattern was observed to suggest a strong impact of FSHR-29 on embryo quality, (p = 0.88 for GI, p = 0.40 for GII).

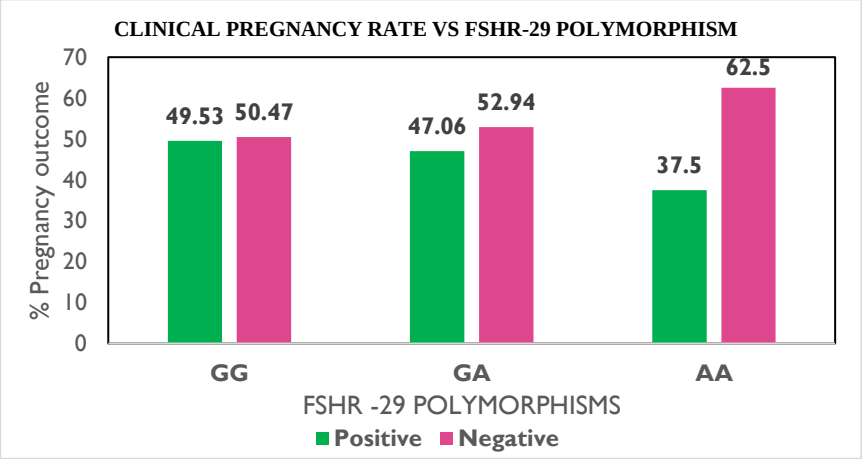

Fig 3. Clinical Pregnancy Rate Vs FSHR -29 Polymorphism

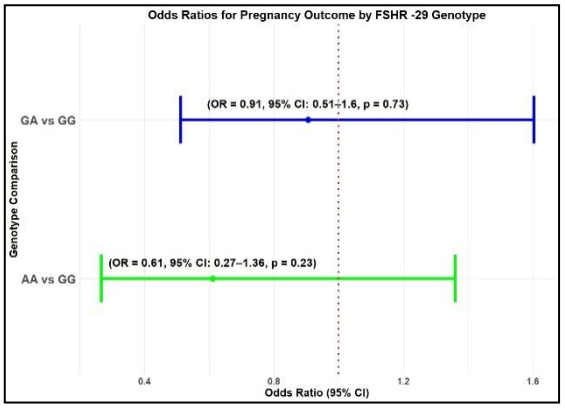

Fig 4. Odd ratio plot for clinical pregnancy rate vs FSHR-29 polymorphism (GG taken as reference)

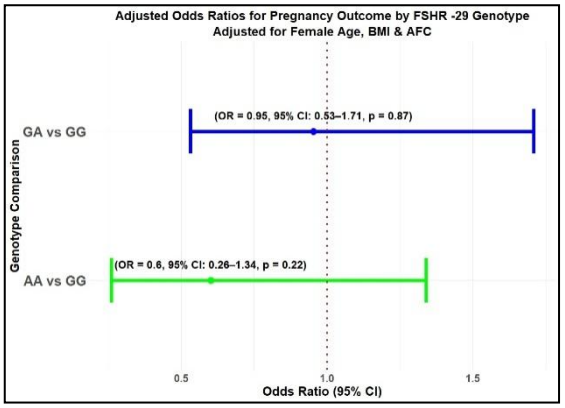

Fig 5. Adjusted for Female Age, BMI, AFC Odd ratio plot for clinical pregnancy rate vs FSHR-29 polymorphism (GG taken as

The analysis of clinical pregnancy rates revealed that the highest success was observed in individuals with the GG genotype (49.53%), followed by GA (47.06%), and the lowest success rate was found in those with the AA genotype (37.5%). This trend suggests a potential association between the FSHR -29 polymorphism and reduced reproductive success, particularly in AA carriers. However, logistic regression analysis with unadjusted odds ratios indicating no significant difference in pregnancy likelihood between GA vs. GG (OR = 0.906, 95% CI: 0.511–1.602, p = 0.73) and AA vs. GG (OR = 0.611, 95% CI: 0.266–1.359, p = 0.23). After adjusting for female age, BMI, and AFC, the odds ratios remained similar (GA vs. GG: OR = 0.953, 95% CI: 0.531–1.708, p = 0.87; AA vs. GG: OR = 0.601, 95% CI: 0.261–1.339, p = 0.22). The Odds ratio plots further confirmed that the confidence intervals for all comparisons crossed the null value (OR = 1), indicating no statistically significant effect of FSHR-29 genotype on pregnancy outcomes. The consistent pattern of lower pregnancy rates in AA individuals aligns with previous research suggesting that FSHR-*29* polymorphism may influence implantation and early pregnancy maintenance.

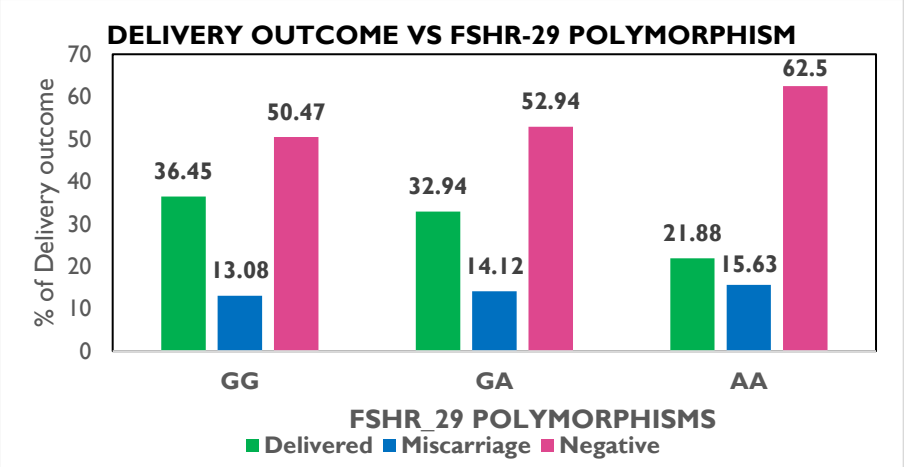

Fig 6. Delivery outcome vs FSHR-29 polymorphism

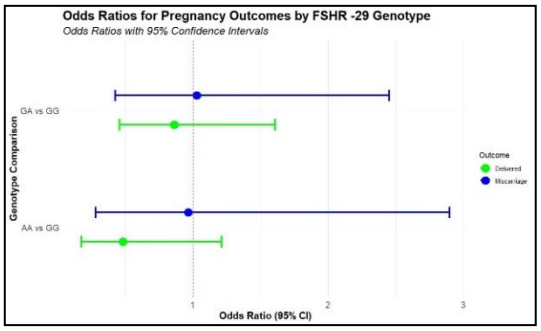

Fig 7. Odds Ratio plot for Deliveries vs. Negative by FSHR-29 Genotype (GG as Reference)

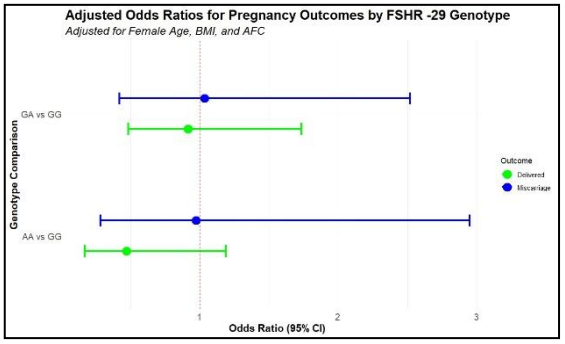

Fig 8. Adjusted for Age, BMI and AFC, Odds Ratio plot for Deliveries vs. Negative by FSHR-29 Genotype (GG as Reference)

The trend of reduced reproductive success in AA carriers was further reflected in delivery outcomes, where the AA group had the lowest delivery rate (21.88%) and the highest negative pregnancy rate (62.5%), suggesting a possible association between *FSHR-29* polymorphism and lower live birth rates. GA vs. GG: OR = 0.862, p = 0.641; AA vs. GG: OR = 0.485, p = 0.14, the pattern suggests that AA individuals may have a higher predisposition to implantation failure or early pregnancy loss. After adjusting for confounders, the odds ratios remained similar (GA vs. GG: OR = 0.918, 95% CI: 0.484–1.738, p = 0.79; AA vs. GG: OR = 0.473, 95% CI: 0.171–1.190, p = 0.13), indicating that *FSHR-29* genotype does not significantly influence live birth rates.

Similarly, miscarriage rates were comparable across genotypes, with no distinct trend observed in GA or AA groups relative to GG. Given the previously established link between *FSHR* polymorphisms and ovarian response variability, it remains plausible that *FSHR-29* could indirectly contribute to early pregnancy maintenance through its effects on follicular recruitment and endometrial receptivity and its extragonadal effect on endometrium. However, logistic regression analysis did not indicate a significant association between *FSHR-29* and miscarriage risk (GA vs. GG: OR = 1.029, p = 0.95; AA vs. GG: OR = 0.96, p = 0.950), with adjusted ORs showing similar results (GA vs. GG: OR = 1.036, 95% CI: 0.421–2.522, p = 0.94; AA vs. GG: OR = 0.975, 95% CI: 0.284–2.948, p = 0.97).

4. DISCUSSION

The FSHR-29 polymorphism, a single nucleotide variation in the promoter region of the FSHR gene, plays a crucial role in regulating gene expression and receptor protein synthesis. This polymorphism can alter transcription factor binding affinity, reducing gene transcription and leading to lower mRNA production. Consequently, fewer FSH receptors are synthesized, limiting their availability on target cells like ovarian granulosa cells. This reduction impairs FSH signaling, affecting key reproductive processes such as follicular development in females. These molecular-level changes likely contribute to the observed differences in gonadotropin sensitivity, pregnancy outcomes, and ovarian response among different FSHR-29 genotypes, reinforcing the need for further studies to validate these associations and explore their clinical implications.

This Study aimed to explore the influence of FSHR-29 polymorphism on various reproductive parameters in PCOD patients

undergoing ART cycles. The observed trends suggest a potential association between FSHR-29 polymorphism and ovarian response, gonadotropin sensitivity, and pregnancy outcomes, particularly in AA individuals. However, statistically significant differences were not observed, indicating that while these trends may be clinically relevant, further research with larger cohorts is needed to establish their definitive significance.

Ovarian Reserve and AFC: The Role of FSHR-29 Polymorphism in PCO patients

Our study showed that GA individuals had a slightly lower mean AFC compared to GG and AA individuals; however, the median AFC was identical across genotypes. These findings suggest that FSHR-29 polymorphism may not play a major role in determining ovarian reserve. Similar observations have been reported in earlier studies, including Desai *et al*. (2011), which found that the AA genotype was associated with lower FSH receptor expression in granulosa cells, potentially leading to reduced follicular recruitment. However, other studies, such as Allegra *et al*. (2017), did not find a strong correlation between FSHR-29 polymorphism and AFC, indicating that the impact of this genetic variant may be modulated by other genetic or environmental factors.

Gonadotropin Sensitivity and Total FSH dose in PCO patients

A significant trend observed in our study was that AA individuals required the highest dose of FSH (2284.94 ± 702.73 IU) compared to GG (1987.85 ±558.48 IU) and GA (1985.88 ± 567.54 IU). This trend aligns with findings from Desai *et al*. (2013) and Alviggi *et al*. (2023), both of which reported that AA genotype carriers required higher FSH doses for ovarian stimulation due to reduced FSH receptor expression and signaling efficiency. The reduced sensitivity to exogenous FSH in AA carriers is likely due to altered receptor transcription and lower expression levels in granulosa cells, leading to suboptimal FSH action during ovarian stimulation.

Oocyte yield and FSHR-29 Influence in PCO patients

Our study found that oocyte yield was comparable across genotypes, with a median of 20 oocytes in all groups. However, the AA group had a slightly higher mean number of retrieved oocytes (18.41±3.68) compared to GG (18.09±3.03) and GA (17.87±3.6) in PCO patients. This is in contrast with Desai *et al*. (2011) (sample size 100) and Allegra *et al*. (2017), whose study population were general infertile women, found that AA carriers tend to have a lower oocyte yield due to reduced ovarian response to gonadotropin stimulation in general infertile population. The discrepancies between studies highlight the complex interplay between genetic variants and individual ovarian physiology.

Embryo Quality and FSHR-29 Genotype Variability in PCO patients

Our findings suggested some variability in embryo quality among genotypes, with GA individuals having a higher proportion of Grade II embryos, while AA individuals exhibited a slightly higher proportion of Grade I embryos. This aligns with the findings of Alviggi *et al*. (2023), which indicated that FSHR-29 polymorphism does not significantly affect embryo quality. Nevertheless, lower FSH receptor expression in AA individuals may contribute to differences in follicular development and oocyte competence, potentially influencing embryo developmental potential.

Pregnancy and Delivery Outcomes: FSHR-29 and Reproductive Success in PCO patients

A consistent trend was observed where GG individuals had the highest clinical pregnancy rates (49.53%), followed by GA (47.06%) and AA (37.50%). The lower pregnancy rates in AA individuals suggest a potential association between FSHR-29 and implantation or early pregnancy maintenance. Prior studies, including Desai *et al*. (2013) and Alviggi *et al*. (2023), have reported similar trends, where AA carriers exhibited lower pregnancy rates, potentially due to compromised follicular recruitment, lower oocyte quality, or suboptimal endometrial receptivity.

Similarly, AA individuals had the lowest delivery rate (21.88%) and the highest negative pregnancy rate (62.5%), reinforcing the trend of reduced reproductive success in this group. These findings are in agreement with previous literature, such as the study by Allegra *et al*. (2017), which reported that FSHR-29 AA carriers exhibited lower implantation rates and reduced live birth outcomes in ART cycles.

Miscarriage Rates and FSHR-29 Genotype in PCO patients

Our study found no distinct trend in miscarriage rates across genotypes, consistent with the findings of Alviggi et al. (2023), who reported that the FSHR-29 polymorphism is not strongly associated with miscarriage risk. However, given that FSHR polymorphisms influence follicular recruitment and ovarian response, along with their extragonadal effect on the upregulation of endometrial steroidogenic gene expression (Sacchi, S., et al.,2018), it is possible that they may have effects on early pregnancy maintenance, warranting further investigations.

The odds ratio plot confirmed that the observed odds ratios for pregnancy and delivery outcomes followed a consistent trend, with AA individuals showing lower odds of live birth and higher odds of negative outcomes. This visualization supports previous findings from Desai *et al*. (2011) and Alviggi *et al*. (2023), which highlighted similar patterns in reproductive outcomes among different FSHR-29 genotypes.

5. CONCLUSION

This study investigated the impact of the FSHR-29 polymorphism (rs1394205) on ovarian response, gonadotropin sensitivity, and reproductive outcomes in PCOD patients undergoing ART cycles. The findings suggest that AA genotype carriers required higher gonadotropin dosages for ovarian stimulation, indicating reduced FSH sensitivity. Additionally, clinical pregnancy and live birth rates were lower in AA carriers, while negative pregnancy outcomes were higher, pointing to a potential genetic influence on implantation and early pregnancy maintenance.

While these trends suggest a possible association between FSHR-29 polymorphism and reproductive success, no statistically significant differences were observed in clinical pregnancy and live birth rates across genotypes. This underscores the need for larger, well-powered studies to confirm these findings and establish their clinical relevance.

Limitations:

- Being a single-center study, its findings may not be generalizable to broader populations. It also does not account for potential ethnic variations in how FSHR-29 polymorphisms affect ovarian response.
- The cohort size (224 PCOD patients) may limit statistical power, necessitating larger studies for validation and subgroup analyses. Additionally, no molecular assays, such as FSH receptor expression analysis in granulosa cells, were conducted to validate underlying biological mechanisms.
- Statistical adjustments were made for age, BMI, and AFC, other potential confounders like metabolic profile, insulin resistance, and lifestyle factors were not addressed.

*Future Prospectives:*Multi-centric globalstudies are required to confirm the observed associations and explore potential interactions with other genetic and environmental factors. Functional studies are needed to elucidate the mechanisms by which the FSHR -29 polymorphism might influence ovarian function and IVF outcomes. Apart from -29 promoter polymorphism, finding the interaction with other gene variants also can help in elucidating information related to role of -29 (G>A) promoter variant in IVF outcome in PCOD subjects.

Conflict of interest

This article does not have any possible conflicts of interest

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