PERINATAL JOURNAL

Original Article

Perinatal Journal 2025; 33(1):438-448

https://doi.org/10.57239/prn.25.03310050

# Amphirogulin growth factor level in blood serum and follicular fluid consider as a predictor marker for positive pregnancy out come in women underwent ICSI protocol

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**Abstract**

In female fertility, Amphiregulin is induced by the luteinizing hormone (LH) surge, promoting oocyte maturation and follicular rupture. It supports granulosa cell proliferation and steroidogenesis, essential for the ovulatory process. Evolution if Amphiregulin growth factor level in follicular fluid and blood serum can consider as a predictor marker for oocyte maturation and positive pregnancy rate in women underwent ICSI cycle.This study comprised 50 women with a mean age of 32.14 ± 0.84 years, who attended the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University, Baghdad, Iraq. They participated in the ICSI program from October 2024 to February 2025. All women underwent ovarian stimulation with antagonist protocol and received recombinant follicle stimulating hormone. At the cycle day two, blood sample was collected for infertility hormone assessment and at day of oocyte retrieval, blood sample and follicular fluid were collected for Amphiregulin growth factor estimation using Elisa. Oocytes quality was evaluated. The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft office 2010. The results showed that a solitary positive correlation between follicular fluids amphiregulin growth factor with number of metaphase II oocytes (r=0.800& p=0.003). Follicular fluids amphiregulin growth factor levels were significantly higher among pregnant females (475.55 ± 26.29 vs. 412.30 ± 13.71; p=0.031); however, there was no significant difference of serum amphiregulin growth factor between pregnant and non-pregnant females (p=0.832). ROC curve was used to calculate follicular fluids amphiregulin growth factor as a predictor of positive pregnancy with acceptable sensitivity and cut-off value was ≥ 433.04 pg/ml. The results of current study concluded that high level of Amphiregulin growth factor in follicular fluid can be used as an indicator for oocyte quality and positive pregnancy rate in female undergoing Intracytoplasmic sperm injection protocol.

**Keywords:** Infertility, Amphiregulin growth factor, Follicular fluid, Intracytoplasmic sperm injection, Pregnancy rate.

## Introduction

The assisted reproductive technologies (ART) provide couples who suffer from infertility with hope to have a normal live birth. In vitro fertilization (IVF) together with intracytoplasmic sperm injection (ICSI) manipulates gametes and embryos in laboratories for conception purposes [1], [2]. Controlled ovarian stimulation (COS) protocols use stimulation protocols to collect maximum mature oocytes which increases the success rates in ART cycles [3], [4], [5], [6].

Reproductive medicine strives to identify the perfect reproductive cycle as an essential objective, however achieving the best selection method remains challenging. The definition of helpful non-invasive indicators needs to be established for predicting intracytoplasmic sperm injection (ICSI) outcomes.

Research on follicular fluid (FF) indicators to predict outcomes has increased in recent years but scientists continue to raise objections. The investigation of potential follicular regulators now incorporates researchers' attention towards epidermal-like growth factor (EGF) family members in addition to established reproductive hormones which have received analysis for over ten years [7], [8]. Several reproductive processes such as follicular development and oocyte maturation processes could be affected by epidermal growth factor (EGF), Amphiregulin (AREG), betacellulin (BTC), and epiregulin (EREG) based on existing research [9], [10,48]. The Epidermal Growth Factor family member AREG functions as an essential substance for cellular growth and reproductive health while helping cells multiply and heal tissues. The two forms of Amphiregulin exist as a truncated version that includes 78 amino acids and a longer form extending six amino acids from its amino-terminus. Within

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AREG's hydrophilic amino-terminal section the lysine and arginine and asparagine residues work together to improve its attachment to the Epidermal Growth Factor Receptor (EGFR) [11,47]. The hormone LH causes Amphiregulin release that leads to oocyte maturation and the rupture of the follicles in women according to [10], [12]. The compound enables granulosa cell proliferation along with steroidogenesis that directs the ovulatory cycle. Pregnancy success and implantation effectiveness depend on AREG's capability for improving endometrial receptivity according to [13], [14]. Investigations into Amphiregulin together with other growth factors have shed light onto their functions in ovarian development and ovulation and implantation processes. The intricate nature of infertility requires specific treatments because these factors disperse improperly in medical conditions including PCOS and endometriosis [14], [15].

The research established follicular fluid Amphiregulin growth factor evaluation for determining oocyte maturation predictions in infertile patients receiving (ICSI) treatments. The analysis between Amphiregulin and reproductive hormones aims to establish its value as a biomarker for improved performance of assisted reproductive technologies (ARTs). The research explores the relationship between AREG concentrations and favorable pregnancy results which supports the creation of specific therapeutic approaches to enhance ART success rates

## Materials and Methods

Fifty infertile females participated in the cross-sectional study with a mean age of 32.14 ± 0.84 years at Assisted Reproductive Technology (ART) programs until they entered the ICSI program at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies located at Al-Nahrain University (Baghdad / Iraq) from October 2024 to February 2025. This research approved by the ethical committee at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies with code number 0702-MF-2025Z48. The mean body mass indices were 29.23 ± 0.59; Each couple had the fertility center's basic fertility work-up, which consists of a physical examination, ovulation detection, evaluation of the uterine and tubal cavities, semen analysis, and case history. The morphological assessment of oocytes extracted from women who are infertile. All patients serological test was done.

### Samples collection

### Blood sample

The (5ml) blood sample collected from all women enrolled in this study at day cycle 2, for hormonal assessment including (LH, FSH, AMH, prolactin and E2) using minividas device.

Moreover (5ml) blood sample collected from capital veins of all women at day of oocyte retrieval for amphiregulin growth factor levels assessment, using Elisa kit (Diagnostic/ USA).

### Follicular fluid collection

At the day of oocyte retrieval from all women enrolled in present study and enter ICSI program, 10 ml of follicular fluid, centrifuge at 2000 rpm for 10 minutes then take supernatant placed in Eppendorf tube which stored at (-20 Cº) and then use for measurement of amphiregulin growth factor levels by Elisa kit (Diagnostic/ USA).

### Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft Office 2010 were used to analyze the data. The data was described using descriptive statistics such as frequency, range, mean, and standard error. Independent sample t-test (unpaired t-test between two groups) and analysis of variance (ANOVA for comparison of more than two groups) were used to compare the groupings. Using Pearson's correlation coefficient (r), the degree of relationship between continuous variables was determined. The receiver Operative Characteristic curve (ROC curve) was used to compute sensitivity, specificity, and cutoff value. Results were deemed statistically significant when the p-value was 0.05 or below.

## Results

Fifty infertile females were enrolled in the present study; the results were expressed in mean plus minus standard error of the mean (SEM). The mean patients' age was 32.14 ± 0.84; thirty-one (62.0 %) females were aged bellow 35 years and nineteen females

(38.0 %) were aged equal to or above 35 years old.

The mean body mass indices were 29.23 ± 0.59; five females (10.0 %) had normal weight, twenty-five females (50.0 %) were over weighted and 20 females (40.0%) were obese. The mean duration of infertility was 7.62 ± 0.72 years ranging from one and half year to twenty-two years (Table 1).

**Table 1.** Baseline characteristics of patients involved in the present study

| **Parameters** | | **Range** | **Mean ± SE** |
|---|---|---|---|
| Age (years) | | 20 – 40 | 32.14 ± 0.84 |
| BMI (Kg/m²) | | 20.2 – 40.0 | 29.23 ± 0.59 |
| Duration of infertility (years) | | 1.5 – 22.0 | 7.62 ± 0.72 |
| **Parameters** | | **N. (%)** | |
| Age groups | < 35 years | 31 (62.0 %) | |
| | ≥ 35 years | 19 (38.0 %) | |
| BMI ranking | Normal weight | 5 (10.0 %) | |
| | Over weight | 25 (50.0 %) | |
| | Obese | 20 (40.0 %) | |
| Type of infertility | Primary | 42 (84.0 %) | |
| | Secondary | 8 (16.0 %) | |

SE: Standard error; BMI: Body mass index; N.: Number of patients, PCOS: poly cystic ovaries

Regarding the ICSI parameters; the mean total oocytes was 12.52 ± 1.19, mean germinal vesicles was 2.58 ± 0.52, mean MI 0.80± 0.18, MII 7.82 ± 0.88 and mean abnormal oocytes 1.32 ± 0.34 follicular fluid amphiregulin growth factor levels were 427.76 ± 12.72. Table 2.

**Table 2.** Baseline ICSI parameters of patients enrolled in the present study

| **ICSI outcome** | **Range** | **Mean±SE** |
|---|---|---|
| Total oocytes count | 2 - 36 | 12.52 ± 1.19 |
| Germinal vesicles (GV) | 0 - 15 | 2.58 ± 0.52 |
| Metaphase I (MI) | 0 - 5 | 0.80 ± 0.18 |
| Metaphase II (MII) | 0 - 26 | 7.82 ± 0.88 |
| Abnormal oocytes | 1 - 12 | 1.32 ± 0.34 |

Serum and follicular fluid amphiregulin growth factor levels were 448.23 ± 15.49 and 427.76 ± 12.72 respectively as demonstrated in table 3.

**Table 3.** Baseline levels of serum and follicular fluids amphiregulin growth factor

| **Parameters** | **Range** | **Mean ± SE** |
|---|---|---|
| Serum amphiregulin growth factor (pg/ml) | 278.82 – 760.53 | 448.23 ± 15.49 |
| Follicular fluids amphiregulin growth factor (pg/ml) | 227.77 – 680.68 | 427.76 ± 12.72 |

SE: Standard error

## Positive pregnancy rate of patients enrolled in the present study

Out of 50 females, 11females were had positive pregnancy test (Pregnancy rate = 22.0 %)

## Follicular fluids amphiregulin growth factor levels as a predictor of positive pregnancy.

A Logistic Regression model was conducted and showed that the Amphiregulin Growth Factor in FF is significantly related to the pregnancy status, X2 (2, N=50) =1.01, p= 0.023), where the likelihood of being pregnant is 1% for every unit increase of Amphiregulin Growth Factor (AREG) in FF (OR=1.01,95% CI [1.001,1.01]). Receiver Operative Characteristic curve (ROC curve) was used to calculate follicular fluids Amphiregulin Growth Factor (AREG) as a predictor of positive pregnancy with overall accuracy of the test is 80%, AUC (Area Under Curve) is 77%, and Cutoff value for AREG FF 434.59. The Sensitivity is 54.55 % (95% CI 23.38% to 83.25%) represents the proportion of observations that are predicted to be positive when the women are truly pregnant. The Specifity is 87.18% (95% CI 72.57% to 95.70%) represents the proportion of observations that are predicted to be negative when the women are non-pregnant. Table 4.

**Table 4:** The Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of using the AREG FF as a predictive diagnostic test for pregnancy among women enrolled in ICSI program.

| Statistic | Value | 95% CI |
|---|---|---|
| Sensitivity | 54.55% | 23.38% to 83.25% |
| Specificity | 87.18% | 72.57% to 95.70% |
| Positive Predictive Value | 54.55% | 31.05% to 76.18% |
| Negative Predictive Value | 87.18% | 77.88% to 92.93% |
| Accuracy | 80.00% | 66.28% to 89.97% |

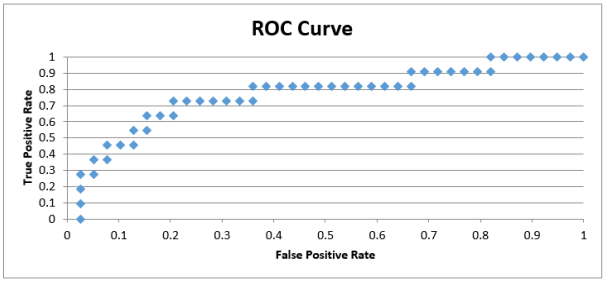


**Figure 2**: ROC curve representing the discriminatory ability of follicular fluids Amphiregulin Growth Factor (AREG) to predict positive pregnancy

Amphiregulin Growth Factor AREG in FF is not correlated to the AREG Serum among the pregnant women, while the AREG in FF is positively correlated with the AREG Serum among the non-pregnant women (r =0.33, p=0.033).

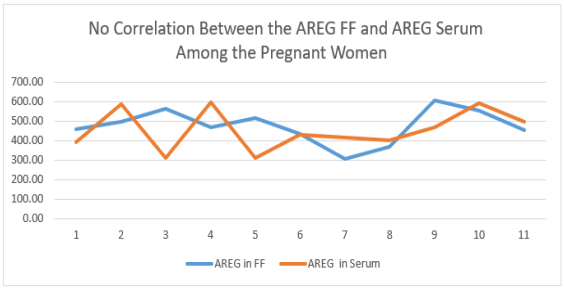


**Figure 3.** The Pearson Correlation test between Amphiregulin Growth Factor (AREG) in serum and follicular fluid among the pregnant women (A).

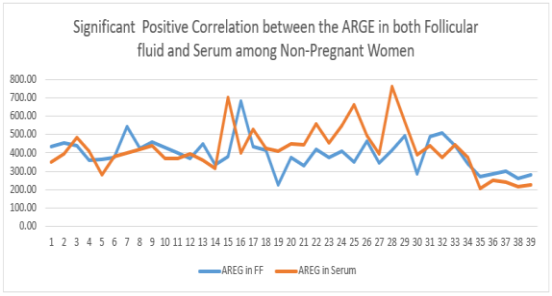


**Figure 4:** The Pearson Correlation test between Amphiregulin Growth Factor (AREG) in serum and follicular fluid among non-pregnant women (B).

## Association between amphiregulin growth factor and predictor (independent) variables

There was a significant association between serum amphiregulin growth factor AREG and body mass index BMI among non- pregnant women, where one unit increase in BMI will associate with an increase of 10.49 pg/ml in the level of serum AREG.

The total oocytes number has a significant association with the level of AREG FF, where the increase of one of the total oocytes associated with an increase of the AREG FF level by 11.33 pg/ml (t= 4.47, P=0.0028, b coefficient= 11.33) as shown in table 4.

The Abnormal oocyte number has a significant negative association with the level of AREG FF, where the increase of one of the abnormal oocytes associated with a decrease of the AREG FF level by

21.18 pg. (t=-7.09, p=0.00019, b coefficient=-21.18). Also, the M2 number has a significant association with the level of AREC FF, where the increase of one of the M2 associated with an increase of the AREC FF level by 15.20 pg/ml (t= 4.19, P=0.003, b coefficient= 15.

**Table 4.** Testing the association of serum and follicular fluid AREG levels with the predictor (independent) variables among pregnant and non-pregnant women

| **Predictor Variable** | **Non-Pregnant Cases** | | **Pregnant Cases** | |
|---|---|---|---|---|
| | AREG FF (P value) | AREG SERUM (P value) | AREG FF (P value) | AREG SERUM (P value) |
| Age | 0.65 | 0.07 | 0.16 | 0.37 |
| BMI | 0.54 | 0.04 | 0.54 | 0.39 |
| Total Oocytes | 0.96 | 0.90 | 0.0029 | 0.85 |
| Abnormal Oocytes | 0.90 | 0.83 | 0.0002 | 0.48 |
| GV | 0.43 | 0.17 | 0.0744 | 0.34 |
| MI | 0.50 | 0.30 | 0.235 | 0.17 |
| MII | 0.79 | 0.92 | 0.003 | 0.83 |

*Multiple Linear Regression; Significant (p≤0.05)

## Discussion

Understanding the relationships between the various possible causes of infertility, such as hormone production, inherited factors, factors that regulate the expression of genes associated to fertility, and altered epigenetic systems, may help identify the reasons of infertility. One of the biggest challenges in reproductive medicine is still identifying possible biomarkers that affect the two most important outcomes of ICSI: the rate of oocyte maturity and pregnancy status.

The purpose of this study was to find the correlations between amphiregulin growth factor with ICSI outcome between pregnant and non-pregnant females. The mean age of the participants in this study was 32.14 ± 0.84 years, with the majority (62.0%) under 35 years. The research conducted by [16] shows 30-35 years is the most common age for patients to access infertility treatments because people today understand reproductive health better and delay having children. Women aged 35 years and above make up 38.0% of patients who seek infertility treatments primarily because of the association between maternal age and reduced fertility rates from diminished ovarian reserve according to [17], [18] and other research investigations have shown that deteriorating ovarian reserve causes adverse outcomes from ART procedures. The findings in this study align with these results, where younger females (<35 years) comprised many participants.

Results showed a mean BMI level of 29.23 ± 0.59 kg/m$^2$ and obesity affected 40.0% of participants who were also overweight at 50.0%. The data demonstrates that BMI elevation creates a direct link with fertility problems. Medical studies have confirmed that obesity leads to disrupted ovulation while creating hormonal imbalance problems and decreasing the results of assisted reproduction techniques [7]. These results are comparable to a recent study by [19], which found that 45% of infertile females undergoing ICSI were overweight or obese. Interventions to optimize BMI before ART cycles have been strongly recommended to improve pregnancy outcomes. The high prevalence of overweight and obesity in this study is consistent with [20], who reported that increased BMI negatively affects ovarian responsiveness and reduces ART success rates.

The mean infertility duration of 7.62 ± 0.72 years observed in this study emphasizes the chronic nature of infertility in many patients. Research in areas marked by cultural and social stigma for infertility shows this detection period matches findings [8].

The research revealed primary infertility occurred in 84.0% of cases compared to secondary infertility which registered at 16.0%. Global patterns show that untreated reproductive tract infections along with lifestyle factors cause primary infertility primarily in resource-limited areas according to [21].

A total of 12.52 ± 1.19 oocytes were retrieved on average from women with a variation of 1 to 36 oocytes. Research literature indicates that pregnancy rates increase when the number of retrieved oocytes reaches higher numbers in ART cycles [22]. Research has demonstrated that this oocyte retrieval number falls within the safest protection zone which leads to better pregnancy outcomes without causing OHSS [23,49]. Studies indicate that an ideal number of oocytes falling between 8-15 pieces during a cycle will deliver the best live birth possibilities alongside minimal medical complications risks [24,46]. Analyzing the mean value of this study shows equivalent results to the observed findings regarding ovarian stimulation protocols.

Research showed that germinal vesicle (GV) oocytes existed in 2.58 ± 0.52 mean values along with 0.80 ± 0.18 metaphase I (MI) oocytes. Any oocyte cohort should naturally incorporate GV and MI oocytes because they are in development stages. High numbers of immature oocytes might signal that ovarian stimulation has been subpar or that the oocytes exhibit inherent quality problems. According to [25] emphasized that embryonic development is restricted in immature oocytes so MII oocytes require priority in research according to their study.

A total of 7.82 ± 0.88 mature MII oocytes were obtained in the study while data ranged from 0 to 26 oocytes. MII oocytes serve as the vital requirement for both successful fertilization and embryogenesis because only this developmental stage can undergo normal post-ICSI fertilization. The study results matched previous research which showed that reaching 6 to 12 MII oocytes results in higher pregnancy success rates [26]. Inside this group the number of MII cells shows variability because each person's ovaries respond differently and because the patients' age and ovarian health levels vary. This research study confirmed that its data matches industry standards for ICSI procedure oocyte collection and assessment during ICSI treatments. The number of retrieved oocytes and MII mature oocytes from this study corresponds to measurements recorded by [27] in their controlled ovarian stimulation patient cohort. The study's increased numbers of abnormal oocytes could possibly result from minor differences between patient characteristics or ovarian stimulation techniques.

The studied population demonstrated a mean value of abnormal oocytes as 1.32 ± 0.34 which ranged between 1 to 12 abnormalities. The premature aging of eggs leads to abnormal oocytes with deformed structures and disturbed cellular content which reduces their fertility potential. Study by [28] confirmed that abnormal oocytes appearing in ICSI cycles create negative effects on both fertilization success rates and embryo development qualities. The quality of oocytes affected by PCOS and obesity demonstrates adverse effects on fertilization and embryonic development according to [20].

Different studies show that amphiregulin growth factor (AREG) exists at 448.23 ± 15.49 pg/ml in serum and 427.76 ± 12.72 pg/ml in follicular fluid to support ovarian function together with intracytoplasmic sperm injection (ICSI) outcomes. Amphiregulin serves as a fundamental member of the epidermal growth factor family to advance follicular development and to help the oocyte mature for luteinization [14]. The study results adhere to [29] as they propose AREG serum measurements serve as biomarkers for ovarian stimulation response. A higher expression of AREG protein in FF suggests improved conditions within follicles which support the maturation process of oocytes and embryonic development.

The research by [9] also supported these findings by showing that elevated AREG levels in follicular fluid result in superior oocyte quality together with enhanced fertility rates which illustrates the importance of follicular microenvironment development. FF AREG concentration differs from serum by being slightly lower because of localized ovarian metabolic processes as observed by [30] who demonstrated systemic and local AREG regulatory differences. Medical research has established amphiregulin as a strong indicator for ovarian responsiveness and ICSI outcome success potential which supports its case as a beneficial biomarker for improving assisted reproduction technology protocols.

Among the 50 females, 11 women succeeded in achieving pregnancy based on the 22.0% pregnancy rate observed throughout this study. Data demonstrates a pregnancy success rate of 22.0% which matches the findings from multiple assisted reproductive technology studies using

intracytoplasmic sperm injection (ICSI) because pregnancy outcomes from 20-30% per cycle depend on age and ovarian reserve status along with embryo quality [31], [32]. While the pregnancy rate in this study falls within the lower range of expected outcomes, it reflects the inherent challenges in treating infertility, especially among women with conditions like polycystic ovary syndrome (PCOS) and obesity, both of which were prevalent in this cohort [33]. A study by [34] emphasized that women with PCOS undergoing ART cycles often experience lower pregnancy success rates due to poor oocyte quality and endometrial receptivity issues. Age and BMI alongside ICSI parameters influence pregnancy rates during pregnancy and most participants fell into the category of being under 35 years old at 62.0% because better pregnancy results are commonly achieved in this age bracket because younger women typically show improved ovarian reserves and better-quality oocytes. Multiple research studies demonstrate that maternal age directly influences pregnancy success because aging tissue decreases oocyte quality while raising chromosomal irregularities [35]s The study showed that obesity and overweight affected most participants since 90% of females exceeded normal BMI values. Elevated BMI causes lower pregnancy success in ART cycles because it damages ovarian functioning and disturbs hormone equilibrium and reduces endometrial receptivity [20]. According to [36], ICSI patients with obesity achieved lower clinical pregnancy rates of 19.6% than women with normal BMI who achieved 30.8% outcomes. The outcome of pregnancy depends heavily on both the maximum number of collected oocytes and the most advanced metaphase II (MII) oocytes. Research findings by [2] show that 7.82 ± 0.88 MII oocytes match their optimal pregnancy rates results.

The research established much higher amphiregulin growth factor levels in FF (fortilizing fluid) from pregnant women when compared to non-pregnant women (475.55 ± 26.29 pg/ml versus 412.30 ± 13.71 pg/ml; p = 0.031). Amphiregulin, a member of the epidermal growth factor (EGF) family, plays a critical role in cumulus-oocyte complex communication, follicular maturation, and oocyte developmental competence [14]. The elevated AREG levels in mature follicle granulosa cells improve both oocyte developmental potential and endometrial responsiveness to allow successful embryo implantation [37]. REAG affects both inflammatory processes and growth signaling pathways which create beneficial conditions in the uterus needed for pregnancy. The ability of AREG to support luteinization functions together with progesterone release accelerates pregnancy development [38]. The scientific evidence indicates that amphiregulin helps regulate how luteinizing hormone (LH) influences oocyte maturation to facilitate implantation successfully [14]. High levels of amphiregulin in FF affect both cumulus cell expansion and oocyte meiotic progression which determines pregnancy results and indicates its ability to serve as an oocyte quality and implantation success biomarker. The research conducted by [9], proved elevated amphiregulin levels in FF lead to better treatment results for assisted reproductive technology protocols.

The laboratory analysis did not demonstrate any meaningful difference between amphiregulin serum levels of pregnant females compared to non-pregnant females. The results imply that amphiregulin impacts primarily the follicle environment yet does not reach systemic levels. Studies by [29], plus other research confirm that serum amphiregulin detections cannot precisely measure ovarian function or embryo quality because amphiregulin primarily operates inside ovarian follicles. Research indicates that amphiregulin performs localized functions in follicle development while VEGF along with other systemic growth factors demonstrate enhanced ability to measure ovarian response [39]. Measurement of amphiregulin in FF serum stands important for predicting ART outcomes because researchers did not find significant variations in levels.

The result shows that follicular fluid amphiregulin levels above 433.04 pg/mL confirm pregnancy outcomes at 81.8% success rate with 67.6% accuracy level while generating a 0.745 AUC (p = 0.016). Research indicates that measuring amphiregulin levels would offer valuable diagnostic potential to estimate pregnancy chances following assisted reproductions. Amphiregulin, a member of the epidermal growth factor family, plays a crucial role in follicular development and oocyte maturation. Studies from [9] proved that elevated amphiregulin concentrations enhance important reproductive aspects of fertility treatments because of its prognostic value. A study by [40], revealed that adding amphiregulin to in vitro maturation media

produced better oocyte development into metaphase II stage cells which demonstrates its essential part in oocyte growth.

The identification between pregnancy and non-pregnancy outcomes based on amphiregulin levels demonstrates a moderate accuracy rate through the ROC analysis with an AUC of 0.745. Research from [9] , confirms that amphiregulin holds significant value for reproductive outcomes. The diagnostic system detects most pregnancies correctly yet its ability to identify non-pregnant women remains only average when measuring amphiregulin activity.

The study shows that amphiregulin content in the follicular fluid has a strong positive connection to the number of metaphase II (MII) oocytes ($r = 0.800$, $p = 0.003$). As amphiregulin bonds with the epidermal growth factor receptor pathway it enables cumulus cell expansion and improves oocyte competence in addition to promoting meiotic progression toward the MII stage [41]. Amphiregulin exhibits significant importance in biomarker development because it improves oocyte developmental potential [29]. Faster embryo maturation rates are expected when elevated AREG levels exist in the follicular fluid because it leads to increased mature oocyte numbers that support successful fertilization and embryo development. Researchers have previously established that AREG actively participates in the oocyte maturation process. The researchers at [9], established that follicular fluid levels of AREG directly impact oocyte maturation together with fertilization rates alongside embryo quality during assisted reproduction cycles. The research illustrated how AREG levels rise alongside better oocyte maturation rates which leads to enhanced embryo quality in reproductive systems. The research by [42], revealed how amphiregulin acts as a beneficial factor during human immature oocyte in vitro maturation. The research proved that amphiregulin administration during maturation media improved oocyte outcomes which demonstrates its potential for treating patients in assisted reproductive procedures. The research by [43] revealed that the addition of AREG to in vitro maturation media produced a substantial improvement in the MII stage transition of human germinal vesicle-stage oocytes. The evidence confirms that AREG functions as a major factor behind oocyte maturation advancement.

Laboratory data from intracytoplasmic sperm injection (ICSI) failed to establish a connection between serum AREG levels and total oocytes, metaphase I oocytes, germinal vesicles or abnormal oocytes counts. The different levels of AREG detected in follicular fluid seem to provide a better indicator for monitoring oocyte maturation and embryo quality among patients undergoing ICSI.

The research study establishes that amphiregulin growth factor levels in serum and follicular fluid of non-pregnant females do not correlate significantly with different intracytoplasmic sperm injection (ICSI) parameter measurements. The data shows amphiregulin appears to lack importance in the developmental process of oocytes and their maturation stages in this non-pregnancy condition. The research data demonstrates consistency with former findings that showed amphiregulin expression reaches lower levels in non-pregnant groups versus pregnant groups. The study conducted by [44], showed amphiregulin expression decreased in non-pregnant subjects for both mural granulosa cells and cumulus granulosa cells thus establishing their relationship with conception success rates. The non-pregnant group displayed no meaningful correlations that would link higher amphiregulin levels to better embryo development despite research which has proved their relationship in pregnancy groups. Observations demonstrate the intricate nature of amphiregulin function during reproductive periods since it affects different people in varying ways because of their unique bodily states.

The relationship between amphiregulin growth factor levels and patients' age and body mass index (BMI). Test results show amphiregulin levels remain similar between patients younger or older than 35 and between participants with different BMI levels when measured in serum and follicular fluid. The research indicates amphiregulin expression maintains stable levels throughout all analyzed variables. The research shows that age together with BMI do not affect amphiregulin growth factor levels yet both parameters have established effects on the reproductive system according to [45]. These factors fail to produce significant amphiregulin level variation even though they might affect fertility by alternative mechanisms.

## Conclusion

The researchers established that high levels of Amphiregulin (AREG) growth factor in follicular fluid indicate better oocyte maturation potentials and successful pregnancy rates in women who receive ICSI treatment. Research findings show positive relationship between AREG levels and the number of metaphase II oocytes as major indicator for oocyte competence capability leading to successful fertilization. Research indicates that pregnant females display elevated AREG concentrations than non-pregnant females because AREG shows promise to enhance the success rates of ART procedures. Research evidence demonstrates that AREG plays a vital role in reproductive healthcare along with assisted conception techniques.

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