ORIGINAL ARTICLE

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Ovarian reserve and pregnancy prognosis in vitro fertilization

Reserva ovárica y pronóstico de embarazo en fertilización in vitro

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ABSTRACT

Introduction: The woman's age and ovarian reserve are the most important factors in fertility treatment. Ovarian reserve can be calculated through antral follicle count (AFC) with vaginal ultrasound, providing valuable information on the pregnancy prognosis in assisted reproduction. Objective: To define the significance of AFC in the pregnancy prognosis in in vitro fertilization. Methods: Retrospective observational study in which the clinical records of 508 infertile patients who underwent in vitro fertilization at the Centro de Fertilidad y Ginecología del Sur from December 2005 to April 2024 were reviewed. The clinical pregnancy rate in women with AFC ≥ 5 and in those with AFC ≥ 5 is analyzed and compared with the differences found according to age. Results: The clinical pregnancy rate in women with AFC ≥ 5 was 35.2%, while it was 14.3% in women with AFC < 5 (p = 0.0236), and was 41.9% in patients < 35 years and 27.2% in those ≥ 35 years (p = 0.0005). Differences in clinical pregnancy rate are more transcendent when considering AFC rather than age (Odds Ratio: 3.3 versus 1.9, respectively). Conclusions: Patients with AFC < 5 have three times lower clinical pregnancy rate in IVF than those with greater ovarian reserve.
Key words: Ovarian follicles, Ovarian reserve, fertilization in vitro

RESUMEN

Introducción. La edad de la mujer y la reserva ovárica son los factores más importantes en los tratamientos de fertilidad. La reserva ovárica puede ser calculada a través del recuento de folículos antrales (AFC, por sus siglas en inglés) con el ultrasonido vaginal, proporcionando información valiosa del pronóstico de embarazo en reproducción asistida. Objetivo. Definir la trascendencia del AFC en el pronóstico de embarazo en fertilización *in vitro*. Métodos. Estudio retrospectivo observacional en el que se revisa los registros clínicos de 508 pacientes infértiles que realizaron fertilización *in vitro* en el Centro de Fertilidad y Ginecología del Sur desde diciembre 2005 hasta abril de 2024. Se analiza la tasa de embarazo clínico en las mujeres con AFC ≥ 5 y en aquellas con AFC< 5, y se compara con las diferencias encontradas de acuerdo con la edad. Resultados. La tasa de embarazo clínico en mujeres con AFC ≥ 5 fue 35,2%, mientras esta fue de 14,3% en mujeres con AFC < 5 (p = 0,0236), y fue de 41,9% en las pacientes < 35 años, y de 27,2% en las ≥ 35 años (p = 0,0005). Las diferencias en las tasas de embarazo clínico son más trascendentes al considerar el AFC en lugar de la edad (*Odds Ratio*: 3,3 versus 1,9, respectivamente). Conclusiones. Las pacientes con AFC < 5 tienen tres veces menor tasa de embarazo clínico en fertilización *in vitro* que aquellas con mayor reserva ovárica.
Palabras clave. Folículo ovárico, Reserva ovárica, Fertilización *in vitro*

Introduction

Infertility is diagnosed in 10% of couples. Its causes are distributed between female and male factors defined through diagnostic tests that allow the proposal of treatments and procedures to increase the chances of pregnancy. Low and high complexity assisted reproduction techniques (ART) are performed on the basis of stimulating the ovary with ovulation inducers and achieving multiple eggs susceptible to fertilization.

The response to stimulation is different in each patient, and there may be an excessive response as well as a poor ovarian response (POR) to ovulation inducers. The rate of euploidy depends on the age of the woman, but not on the number of blastocysts achieved[1]. The greater the number of mature oocytes aspirated, the more euploid blastocysts will be achieved. However, the rate of euploidy will be constant and independent of the number of blastocysts achieved[2].

POSEIDON stratification is aimed at integrating and homogenizing the diagnostic criteria for POR in view of the diversity of definitions and the imprecision of the Bologna criteria[3]. This stratification directs the concept of POR towards that of 'low prognosis', seeking to define groups of patients according to egg quality (age) and ovarian reserve (AFC or anti-Müllerian hormone (AMH) dosage), determining success rates and suggesting strategies for each group[4,5]. 13.1% of in vitro fertilization (IVF/ICSI) patients at the Centro de Fertilidad y Ginecología del Sur (CFGS) are within the POSEIDON groups[6], and this proportion could be between 13 and 52% depending on the different centers[7].

The AFC is a variable available to every gynecologist, by visualizing and counting the antral follicles through vaginal ultrasound. The AMH also allows us to know the ovarian reserve; however, it is not widely available, and the result of the analysis may require some days. In controversial situations, in which there is no correlation or coincidence between AFC and AMH, AFC is the variable with the best predictive value[8].

The present study seeks to highlight the importance of AFC in the prognosis of pregnancy in IVF/ICSI.

Methods

A retrospective observational study was performed by reviewing the records of 508 infertile patients who underwent IVF/ICSI at the CFGS, located in the city of Cusco, southern Peru, at 3,330 meters above sea level, between December 2005 and April 2024.

The POSEIDON stratification divides women with POR into 4 groups according to age and ovarian reserve (defined through AFC and AMH)[4]:

- Group 1: age < 35, AFC ≥ 5 or AMH ≥ 1.2 ng/mL and ≤ 9 oocytes aspirated in a previous IVF cycle.
- Group 2: age ≥ 35, AFC ≥ 5 or AMH ≥ 1.2 ng/mL and ≤ 9 oocytes aspirated in a previous IVF cycle.
- Group 3: age < 35, AFC < 5 or AMH < 1.2 ng/mL.
- Group 4: age ≥ 35, AFC < 5 or AMH < 1.2 ng/mL.

The AFC is the ovarian reserve variable recorded in all patients, so we used it in the present study. It was defined through vaginal ultrasound, counting the number of follicles between 2 and 9 mm in diameter in both ovaries, between 2 to 5 days from the onset of menstruation and within 3 months prior to the IVF/ICSI cycle.

Ovarian stimulation was performed using gonadotropin-releasing hormone (GnRH) agonists or antagonists associated with human menopausal gonadotropin or recombinant FSH. Stimulation was monitored by vaginal ultrasound and follicular aspiration was programmed 36 hours after the application of human chorionic gonadotropin (hCG).

Life-Global culture medium was used for gamete manipulation and embryo culture in CO2 or triple gas incubator, with CO2 concentration at 9.0%. Embryo transfer was through a flexible catheter under abdominal ultrasound guidance, on the third or fifth day of culture.

β hCG subunit dosage was performed 17 days after follicular aspiration, and after 10 days a vaginal ultrasound was performed to determine the number of implanted embryos and cardiac activity.

Clinical pregnancy rate (CPR) is defined as the number of patients with active pregnancy relative to the number of patients with embryo transfer.

CPR was analyzed in women with AFC ≥ 5 and in those with AFC ≥ 5 (POSEIDON 3 and 4) and compared with CPR according to age.

The statistical significance of the differences found was analyzed by means of t-tests, Fisher's exact test and Odds ratio calculation.

Results

The general characteristics of patients with AFC ≥ 5 and AFC < 5 were similar in relation to age, parity, history of abortions, number of embryos transferred, and endometrial thickness. Patients with AFC < 5 (POSEIDON groups 3 and 4) showed shorter infertility time, used lower doses of gonadotropin, and achieved lower numbers of metaphase II and fertilized oocytes (Table 1).

Ovarian reserve, represented by AFC, determines a CPR of 35.2% when AFC ≥ 5, and 14.3% when

AFC < 5. Age is related to egg quality and determines a CPR of 41.9% in patients < 35 years and 27.2% in patients ≥ 35 years (Table 2).

Discussion

Age 35 years determines differences in CPR (Table 2), with clinical pregnancy being almost twice as likely in women < 35 years than in older women.

However, AFC may be of greater significance, as women with AFC ≥ 5 are three times more likely to have a clinical pregnancy in IVF/ICSI than women with AFC < 5 or POSEIDON 3 and 4 (table 2). POSEIDON 3 and 4 patients achieved a lower number of metaphase II and fertilized eggs than women with higher ovarian reserve (Table 1).

The distribution of POSEIDON groups and pregnancy prognosis is different according to the populations. Li finds ovarian reserve as a determinant, with POSEIDON groups 3 and 4 with live birth rates (LBR) at 14.7% and 6.6%, respectively, as opposed to POSEIDON groups 1 and 2 with LBR at 56.0% and 30.9%, respectively[9]. Shi's research finds age as a determinant, with POSEIDON groups 1 and 3 or younger women having better NBT compared to POSEIDON groups 2 and 4 or older women[10].

Several studies have suggested alternative treatments and procedures to increase the possibility

Table 1. General and IVF/ICSI cycle characteristics according to AFC in the total group of patients.

	AFC	media	Standard deviation	*p* value
Age	≥ 5 < 5	34.4 37.6	4.4 4.2	0.982
Parity	≥ 5 < 5	0.2 0.3	0.5 0.5	0.107
Abortions	≥ 5 < 5	0.8 1.0	1.0 1.0	0.355
Years of Infertility	≥ 5 < 5	5.1 3.4	3.7 3.0	0.047
AFC	≥ 5 < 5	13.8 3.0	7.0 1.0	0.000
Gonadotropin dose	≥ 5 < 5	2,164.5 1,563.5	552.3 991.3	0.000
Metafase II oocytes	≥ 5 < 5	8.4 2.5	5.0 1.6	0.000
Fertilized oocytes	≥ 5 < 5	6.4 2.3	4.0 1.5	0.000
Transferred embryos	≥ 5 < 5	2.6 1.9	0.8 1.0	0.092
Endometrial thickness	≥ 5 < 5	9.2 8.2	2.0 2.0	0.834

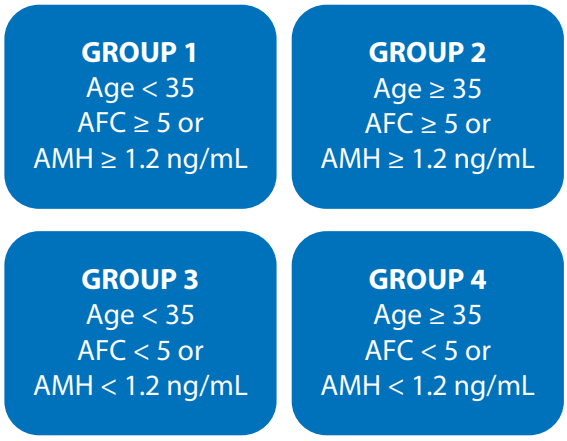
AFC: antral follicle count

Table 2. CPR according to age and AFC.

	CPR	*p* value	OR	CI 95%
Age < 35 Age ≥ 35	41.9% (99/236) 27.2% (74/272)	0.0005	1.9	1.3-2.8
AFC ≥ 5 AFC < 5	35.2% (167/474) 14.3% (4/28)	0.0236	3.3	1.1-9.6

CPR: clinical pregnancy rate; OR: Odds ratio; CPR: clinical pregnancy rate; 95% CI: 95% confidence interval; AFC: antral follicle count

Figure 1. POSEIDON groups.



AFC: antral follicle count; AMH: antimüllerian hormone

of pregnancy in the POSEIDON 3 and 4 groups. The double stimulation protocol (Duo Stim) could be an acceptable alternative, since it obtains oocytes in both follicular and luteal phases[11,12].

The use of GnRH agonists or antagonists[13], low doses[14] or the use of both products in an ovarian stimulation cycle[15], the coadministration of clomiphene citrate and letrozole[16], as well as the use of medroxyprogesterone or didrogesterone[17] have not shown a positive effect on CPR. Administration of platelet-rich plasma into the ovaries has contradictory results, possibly due to heterogeneity in plasma preparation methods, as well as in the selection of POR patients[18,19]. Egg or embryos with the aim of achieving a significant number amenable to transfer (banking) has not demonstrated an increase in CPR[20,21]. The administration of GnRH agonists and hCG for the programming of egg retrieval ('double trigger') also has contradictory results[22,23].

Other alternatives have been evaluated and most researchers recommend not using growth hormone, testosterone, estradiol, aromatase inhibitors, modified natural cycle and microdoses of gonadotropins.

Ovodonation ends up being an option accepted by many women with POR, due to its high CPR and lower cost compared to the investment that could be made with the treatments mentioned above.

The present study provides information that requires further strengthening through a larger number of patients, reducing the confidence

intervals in the statistical analysis. Difficulty in following patients until the birth event precludes expressing the results as live birth rates.

Patients in POSEIDON groups 3 and 4 have three times lower CPR in IVF/ICSI than those with higher ovarian reserve. From the patient's first visit, the gynecologist can have an approximation of the infertile patient's pregnancy prognosis by considering age and AFC, and if the patient is within POSEIDON groups 3 or 4, i.e., has an AFC < 5, she should be given the information of her difficult condition and low pregnancy prognosis with ARTs.

Referencias bibliográficas

1. Ata B, Kaplan B, Danzer H, Glassner M, Opsahl M, Tan SL, Munné S. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. Reprod Biomed Online. 2012;24(6):614–20. DOI: 10.1016/j.rbmo.2012.02.009
2. Colamaria S, Capalbo A, Sapienza F, Maggiulli R, Giuliani M, Gravotta E, Vaiarelli A, Ferrero S, Schimberni M, Giallonardo A, Rienzi L, Ubaldi M. Female age, number of mature eggs and biopsied blastocysts effectively define the chance for obtaining one euploid embryo: counselling and decision-making during PGS cycles S. Hum Reprod. 2015;30:i56. DOI: 10.1093/humrep/30.Supplement_1.1
3. Ferrareti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L; ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of "poor response" to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 2011;26(7):1616-24. DOI: 10.1093/humrep/der092
4. POSEIDON Group (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) Alviggi C., Andersen C. Y., Buehler K., Conforti A., De Placido G., et al., A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. Fertil Steril. 2016;105:1452-3. DOI: 10.1016/j.fertnstert.2016.02.005
5. Roque M, Haahr T, Esteves S, Humaidan P. The POSEIDON stratification – moving from poor ovarian response to low prognosis. JBRA Assist Reprod. 2021;25(2):282-92. DOI: 10.5935/1518-0557.20200100
6. Vargas-Tominaga L, Vargas A, Alarcon F, Medina A, Gómez M, Bejar K. Cumulative live birth rates of patients in the Peruvian Andes according to the POSEIDON criteria: a single-center retrospective analysis. JBRA Assist. Reprod. 2021;25(3):412-6. DOI:10.5935/1518-0557.20200107
7. Vargas-Tominaga L. POSEIDON Stratification. In: Duncan L T (editor). Advances in Health and Diseases. Volume 40. New York. Nova Science Publishers, Inc. 2021:251-64.
8. Hochberg A, Dahan MH, Yarali H, Vuong LN, Esteves SC. Significance of serum AMH and antral follicle count discrepancy for the prediction of ovarian stimulation response in Poseidon criteria patients. J Assist Reprod Genet. 2024;41(3):717-26. DOI: 10.1007/s10815-024-03050-8
9. Li Y, Li X, Yang X, Cai S, Lu G, Lin G, Humaidan P, Gong F. Cumulative Live Birth Rates in Low Prognosis Patients According to the POSEIDON Criteria: An Analysis of 26,697 Cycles of in vitro Fertilization/Intracytoplasmic Sperm Injection. Front Endocrinol (Lausanne). 2019;10:642 DOI: 10.3389/fendo.2019.00642
10. Shi W, Zhou H, Tian L, Zhao Z, Zhang W, Shi J. Cumulative Live Birth Rates of Good and Low Prognosis Patients According to POSEIDON Criteria: A Single Center Analysis of 18,455 Treatment Cycles. Front Endocrinol. 2019;10:409. DOI: 10.3389/fendo.2019.00409
11. Sfakianoudis K, Rapani A, Grigoriadis S, Retsina D, Maziotis E, Tsioulou P, Giannelou P, Pantos K, Koutsilieris M, Vlahos N, Mastorakos G, Simopoulou M. Novel Approaches in Addressing Ovarian Insufficiency in 2019: Are We There Yet? Cell Transplant. 2020;29:963689720926154. DOI: 10.1177/0963689720926154
12. Haahr T, Dosouto C, Alviggi C, Esteves SC, Humaidan P. Management Strategies for POSEIDON Groups 3 and 4. Front Endocrinol (Lausanne). 2019;10:614. DOI: 10.3389/fendo.2019.00614
13. Walker ZW, Lanes A, Srouji SS, Hornstein MD, Ginsburg ES. Ultra-low-dose and very-low-dose Lupron downregulation protocols for poor responders based on POSEIDON group 3 and 4 classifications. J Assist Reprod Genet. 2023;40(8):1881-95. DOI: 10.1007/s10815-023-02842-8
14. Pal A, Mani T, Chinta P, Karthikeyan M, Kunjummen AT, Kamath MS. Effectiveness of GnRH Agonist Short Protocol Versus GnRH Antagonist Protocol in POSEIDON Groups 3 and 4: a Retrospective Cohort Study. Reprod Sci. 2023;30(8):2481-8. DOI: 10.1007/s43032-023-01196-x
15. Orvieto R. Stop GnRH-agonist/GnRH-antagonist protocol: a different insight on ovarian stimulation for IVF. Reprod Biol Endocrinol. 2023;21(1):13. DOI: 10.1186/s12958-023-01069-7
16. Tu X, You B, Jing M, Lin C, Zhang R. Progestin-Primed Ovarian Stimulation Versus Mild Stimulation Protocol in Advanced Age Women With Diminished Ovarian Reserve Undergoing Their First In Vitro Fertilization Cycle: A Retrospective Cohort Study. Front Endocrinol (Lausanne). 2022;12:801026. DOI: 10.3389/fendo.2021.801026
17. Lin HT, Wu MH, Tsai LC, Chen TS, Ou HT. Co-Administration of Clomiphene Citrate and Letrozole in Mild Ovarian Stimulation Versus Conventional Controlled Ovarian Stimulation Among POSEIDON Group 4 Patients. Front Endocrinol (Lausanne). 2022;12:780392. DOI: 10.3389/fendo.2021.780392
18. Najafian A, Alyasin A, Aghahosseini M, Hosseinimousa S, Kazemi SN. Beneficial effects of intraovarian injection of platelet-rich plasma in women with poor ovarian response. Clin Exp Reprod Med. 2023;50(4):285-91. DOI: 10.5653/cerm.2023.06086
19. Barrenetxea G, Celis R, Barrenetxea J, Martínez E, De Las Heras M, Gómez O, Aguirre O. Intraovarian platelet-rich plasma injection and IVF outcomes in patients with poor ovarian response: a double-blind randomized controlled trial. Hum Reprod. 2024;39(4):760-9.DOIi: 10.1093/humrep/deae038
20. Cakmak H. When is the right time to stop autologous in vitro fertilization treatment in poor responders? Fertil Steril. 2022;117(4):682-7. DOI: 10.1016/j.fertnstert.2022.02.027
21. Lee KS, Lin MH, Hwu YM, Yang JH, Lee RK. The live birth rate of vitrified oocyte accumulation for managing diminished ovarian reserve: a retrospective cohort study. J Ovarian Res. 2023;16(1):49. DOI: 10.1186/s13048-023-01128-y

22. Varlı B, Sukur YE, Ozmen B, Sönmezer M, Atabekoğlu C, Aytaç R, Berker B. Age and dual trigger were found to be significant predictors of live birth in POSEIDON group 3 and 4 women treated with the GnRH antagonist protocol: a retrospective cohort study. Eur Rev Med Pharmacol Sci. 2024;28(4):1480-9. DOI: 10.26355/eurrev_202402_35478

23. Keskin M, Ecemiş T, Atik A, Yeğen P, Kalkan E, Yücel GS. Cycle outcomes of dual trigger (GnRH agonist+hCG) versus human chorionic gonadotropin trigger alone in POSEDION group 3-4 poor-responders and normo-responders: A prospective randomized study. J Gynecol Obstet Hum Reprod. 2023;52(8):102633. DOI: 10.1016/j.jogoh.2023.102633