

Evaluation of Ovarian Reserve in Women With Rheumatoid Arthritis

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Abstract

Objective: Subfertility is commonly observed in patients with rheumatoid arthritis (RA). Although the causes are not well established, the alteration of the ovarian reserve is thought to contribute to the lower chances of pregnancy. This cross-sectional study aimed to evaluate the ovarian reserve in patients with RA.

Materials and methods: Two parameters associated with ovarian reserves such as the antral follicle count (AFC) and the anti-müllerian hormone (AMH) were assessed in 38 patients with RA. We also analyzed the correlation of these parameters with the medication used to treat this pathology and with the illness severity.

Results: The AMH levels in women with RA were comparable to those found on healthy individuals although the RA patients were more likely to have a low AFC. Ovarian reserve and RA were neither influenced by parameters of disease activity nor by the use of medication.

Conclusion: The ovarian reserve in women with RA was similar to that found in healthy individuals.

Keywords: Ovarian Reserve; Anti-Mullerian Hormone; Rheumatoid Arthritis

Introduction

The main function of the ovary is to provide mature oocytes that are capable of being fertilized resulting in a healthy and successful pregnancy (1). The number of oocytes during a woman's reproductive

life is finite, therefore the ovarian reserve (OR) is a term used to describe the reproductive potential as a function of the quantity and quality of the remaining oocytes (2). As such, it is one of the most important parameters assessed by clinicians when they evaluate women with fertility problems. One way to assess the OR is through the antral follicle count (AFC) which is obtained by counting the number of small follicles in the ovary with ultrasonography. As for biomarkers,

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the most common test is the measurement of the follicle-stimulating hormone (FSH) and estradiol (1). Importantly, the anti-Müllerian hormone (AMH) is produced by the granulosa cells of growing ovarian follicles and its levels in serum can accurately estimate the OR (3). In contrast to FSH and estradiol that need to be assessed specifically on the 3rd of the menstrual cycle, the AMH levels can be measured at any time due to its stability through the menstrual cycle and the little low variability in subsequent cycles (4).

Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic, autoimmune disease affecting primarily joints of unknown etiology (5, 6). This condition affects ~1% of the population with a higher incidence in the woman of reproductive age (7). The goals of the available treatments are to reduce pain, decrease inflammation, and improve a person's overall functioning preventing joint destruction and deformity (6). The usual treatment for RA includes the use of methotrexate (MTX) which results in significant improvements in ~75% of the patients (8). Female patients experience more fertility problems when compared with healthy individuals, resulting in a longer mean time to pregnancy (9, 10). Also, women with RA usually reach menopause at an earlier time in comparison with healthy individuals (11). Whether the increased time to pregnancy represents a decreased ovarian function is still to be shown. In this regard, little is known about the effect of the disease itself, the inflammation, or the use of antirheumatic drugs on the ovarian reserve. While some studies claim that RA does not affect the ovarian reserve (12) others postulate that this parameter is altered in RA patients (13, 14). A reduced ovarian reserve (ROR) is demonstrated by an AFC of 5-7 follicles or AMH of <1 ng/mL or (15-17).

The role of vitamin D status on the reproductive system has been controversial. The vitamin D has an important role in follicular development, differentiation and luteinization (18) and has been associated with in vitro fertilization (IVF) outcomes and features of other pathologies like polycystic ovarian syndrome (PCOS) and endometriosis in women while improving the semen quality in men (19). Some studies have shown that vitamin D status influences the levels of AMH (20) however, contrasting results have been also reported (21). Additionally, the vitamin D status has also been implicated in the development of autoimmune diseases like RA and its severity (22). Vitamin D has an important role in follicular development,

differentiation and luteinization.

Therefore, the aim of this study was to compare the OR in women with RA with that found in healthy individuals. Also, to correlate the serum vitamin D levels with the ovarian reserve (AMH) levels found in RA patients and controls. Additionally, to evaluate the influence of parameters of disease activity and the medications used for treatment with the ovarian reserve found in RA patients.

Materials and methods

Study design: A cross-sectional and comparative study was performed with patients who attended the Rheumatology service of the Hospital Universitario “Dr. Jose Eleuterio Gonzalez”, and that was diagnosed with RA according to the American College of Rheumatology (ACR). According to the ACR/EULAR criteria, patients who have at least one joint with definite clinical synovitis and whose synovitis is not better explained by another disease (eg. lupus, psoriatic arthritis, or gout) should be tested (23). For comparison, a group of 38 healthy individuals was included as a control.

Patients with a history of endometriosis, Polycystic Ovarian Syndrome (PCOS), hysterectomy, oophorectomy or any other kind of ovarian surgery, pregnant, with primary amenorrhea, or treated for cancer (either with radiotherapy or chemotherapy) were excluded from the study.

Ethical consideration: The study was approved by the Bioethics Committee of the Hospital Universitario “Dr. José Eleuterio González” (#GII5-019). This study complied with the Declaration of Helsinki principles. Patients were provided with written information about the study prior to giving consent.

Clinical measurements: We registered the age and weight of the participants. As for the gynecological history, the number of pregnancies was recorded for RA patients and healthy control individuals. Specifically, for the RA patients, the disease activity score (DAS28) and medications used for treatment were registered. Laboratory measurements for the RA patient group included IgM rheumatoid factor (RF), the anti-cyclic citrullinated peptide (anti-CCP), the erythrocyte sedimentation rate (ESR) and the C- reactive protein (CRP) levels (mg/liter).

Transvaginal ultrasonography and antral follicle count: On the third day of the menstrual cycle, transvaginal ultrasonography was performed using an Apogee 800, L 1500 series (Shantou Institute of Ultrasonic Instruments Co., Ltd. Guangdong, China),

with a 7.5-MHz vaginal transducer. Briefly, while the patient was in a lithotomy position and with an empty bladder, the transducer was advanced about 6 to 8 cm into the vagina angling laterally until the ovary was seen. The length and Antero-Posterior (AP) measurements were obtained in the longitudinal plane, while antral follicles measuring 2–9 mm in diameter were counted and the ovarian volume was calculated in the transversal plane. The total number of follicles in both ovaries were added for the total antral follicle count (AFC).

Measurement of the anti-Müllerian hormone: Blood samples were obtained following an overnight fast. Briefly, blood was drawn into plain serum tubes, the tubes were centrifugated at 3500 rpm for 10 min and the serum was separated and stored at -20°C until further analysis.

AMH serum levels were assessed by using the AMH Gen II enzyme linked immunosorbent assay (ELISA) (Beckmann Coulter, Brea, CA, USA). The AMH Gen II ELISA assay demonstrated stable intra- and inter-assay coefficients of variation of 5% and a functional sensitivity of 0.35 ng/ml.

Measurement of serum vitamin D: The vitamin D level was determined through the Elecsys Vitamin D3 (25OH) electrochemiluminescence immunoassay (ECLIA) following the manufacturer's recommendations. The used cut-off values were reported previously (24).

Statistical analysis: Data are expressed as mean and standard deviation (SD). Mean comparisons were performed by Student T-test or the Mann-Whitney *U* test for a non-parametrical test. For the association of two categorical variables, Fisher's exact test was used. The correlation between variables was analyzed with the Person's or Spearman's correlation tests.

Calculations were performed with GraphPad Prism version 8.4.2 for Windows (GraphPad Software, San Diego, CA, USA). A $p < 0.05$ was considered statistically significant.

Results

Demographic and clinical characteristics of participants: A total of 76 participants were enrolled in the study, 38 of them had RA diagnosis while the remaining 38 were healthy individuals and therefore were used as a control group. There was not a significant difference in the age of both groups ($p=0.96$) with 31.47 ± 5.7 and 31.42 ± 5.43 (mean \pm standard deviation) for RA patients and healthy controls respectively (Table 1).

As for the gynecologic history, there was no difference in the number of pregnancies, cesarean deliveries, miscarriages, or ectopic pregnancies in either the control or RA groups. However, the number of vaginal deliveries were significantly different between the groups ($p=0.01$). Regarding the RA group, the diagnosis occurred 5 years prior to the start of the study and most of them initiated the treatment shortly after their diagnosis, on average 4.7 years before starting the study.

As for the biomarkers used for the RA diagnosis, the RF and anti-CCP mean levels were 197.43 UI/ml and 139.23 UI/ml, respectively. Also, the non-specific inflammation markers were altered with mean values of 30 mm/h for the erythrocyte sedimentation rate and 2 mg/l for the CRP level (Table 1).

The RA patients were categorized by their DAS28 score, for which 5% of the patients ($n=2$) were in remission, 11% ($n=24$) had "low activity", the 63% of the patients ($n=24$) displayed a "moderate activity" and the 21% ($n=8$) showed a disease status of "high activity".

Table 1: Clinical characteristics of control and RA patients

	Control individuals			RA patients			p-value
	Mean (SD)	95% CI	IQR	Mean (SD)	95% CI	IQR	
Age (years)	31.42 (5.43)	29.63-33.21	26.75-35.25	31.47 (5.72)	29.59-33.35	26.75-36	0.96
Weight (Kg)	NA		NA	70.15 (17.07)	63.88-76.41	55.00-83	NA
Time from diagnosis (years)	NA	NA	NA	5.0 (4.0)	3.707-6.398	2.2-5	NA
Time to the initiation of treatment (years)	NA	NA	NA	4.7 (4.0)	3.378-6.043	2-5	NA
Rheumatoid factor (UI/ml)	NA	NA	NA	197.43 (105.9)	162.6-232.3	192.5-200	NA
Anti-citrullinated protein antibody (UI/ml)	NA	NA	NA	139.23 (139.85)	93.27-185.2	29.6-198.5	NA
Erythrocyte sedimentation rate (mm/hr)	NA	NA	NA	30.0 (22.31)	22.75-37.41	18.25-36	NA
C-reactive protein (mg/l)	NA	NA	NA	2.0 (2.9)	1.020-2.984	0.6-2.11	NA

Values expressed as mean (SD). SD= Standard deviation, IQR= Inter-Quartile Range. RA: rheumatoid arthritis.

For their treatment, MTX, leflunomide and sulfasalazine were mainly used. About half (53%) of the patients were treated with two drugs, 39% of them received two drugs and 8% of the patients received monotherapy. The majority (76%) of the RA patients received MTX alone or in combination with other drugs.

Evaluation of the ovarian reserve: To estimate the ovarian reserve, two different approaches were used. The AFC was measured directly by ultrasonography in the RA and control groups and were then categorized by those with an $AFC \leq 5$ and those with an $AFC > 5$ (25). In the control group, 26% of the individuals ($n=10$) had an $AFC \leq 5$ and the remaining 74% ($n=28$) had an $AFC > 5$. In contrast, 52% of the RA patients ($n=20$) had an $AFC \leq 5$ whilst 47% of them ($n=18$) had an $AFC > 5$. The AFC was significantly different between RA patients and healthy individuals ($p=0.03$). There were threefold times chances of having a “low” AFC (<5) for RA patients compared to the healthy individuals in the control group (OR: 3.11, 95%CI= 1.244-7.671). Furthermore, there was not a significant difference between the RA patients with low follicle count ($AFC \leq 5$) that received ≤ 1 year and those with > 1 year of treatment (data not shown).

Additionally, the levels of AMH on serum showed a tendency to be lower in RA patients 2.63 (0.49) however, no significant difference ($p < 0.082$) was found when compared with the levels found in the control group, 2.83 (0.48) (Table 1, Figure 1). There was no correlation of the AMH levels found in RA

patients with their age ($r=0.1240$, $p\text{-value}=0.4583$), weight ($r=0.048$, $p\text{-value}=0.795$) or vitamin D levels ($r=0.079$, $p\text{-value}=0.633$). Overall, we did not find an association between the type of drug used for treatment, the time of treatment initiation, the DAS28 score, the RF and anti-CCP level, ESR, CRP and poor ovarian reserve using the AFC and the AMH levels.

Evaluation of the vitamin D levels in RA patients: The vitamin D serum levels were also measured in RA patients and control individuals and were significantly different when compared ($t=1.90$, $p > t=0.03$) (Figure 1). The vitamin D levels were deficient for the control group and insufficient for the RA patients.

Discussion

Despite many years of data suggesting a higher incidence of infertility in women with RA, the etiology or mechanism is still to be known. Autoimmune pathologies could have an important role in the early exhaustion of the primordial follicle pool and therefore, affect the OR of a woman of reproductive age (26). In the present study, we evaluated the status of the OR in woman with RA and compared it with that of healthy individuals. For that, we used the AFC and the AMH, the most sensitive markers of OR (15). We found that the RA patients had a 3.1 fold more chance to have a low AFC than the control individuals. However, we did not find evidence that the OR is affected in RA patients when we used the AMH levels for comparison.

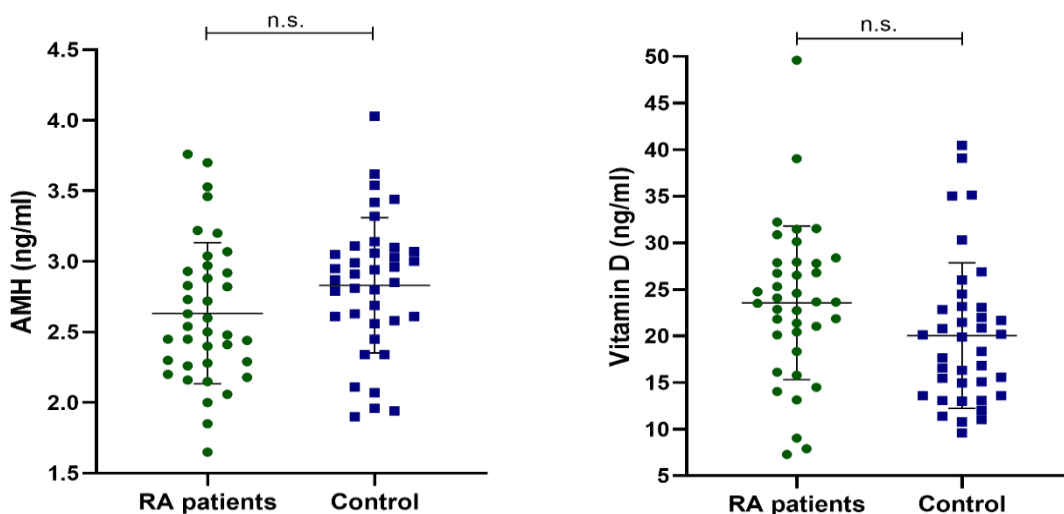


Figure 1: AMH and vitamin D levels in control and RA patients. Dots represent each individual measure, and the lines represent the mean with standard deviation (SD).

The AMH levels found in both groups were above the cut off of ROR (<1ng/ml) consistent with the expected AMH levels for the group of age. Additionally, there was not a significant difference in the number of pregnancies between the RA and control groups therefore there was no evidence of subfertility in the RA patients.

The OR in RA patients has been previously evaluated with contradictory findings. For instance, one study showed that AMH levels in 72 women with early RA were similar to those of healthy controls, suggesting that the reduced fertility was not caused by a diminished ovarian reserve (12). Whilst others have reported lower AMH values on RA patients, concluding that the OR was affected on those patients (13). Similarly, lower AMH levels with respect to control individuals were reported for 75 patients with RA diagnosis (27).

In contrast to the previous studies that used only the AMH levels by ELISA, the inclusion of the AFC by ultrasonography provided evidence that the OR might be affected on RA patients which were not detected by the AMH levels alone. However, our study had a relatively small number of patients, therefore, further studies are required to investigate this finding.

In RA, autoantibodies such as RF and anti- CCP are usually found years before the pathology becomes evident (28) and are thought to represent patients with the highest chance for extra-articular manifestations such as the alteration of the ovarian reserve. In this regard, we did not find a correlation between the presence of autoantibodies and the severity of the disease which was determined by the DAS28 score.

The first- choice medication for diagnosed RA patients is the MTX which has been reported to have a low risk of fertility disturbances (4). However, there is a lack of studies on the effect of low- dose of MTX for long periods of time on OR. For instance, the use of MTX for treatment of ectopic pregnancy has not been associated with a DOR using FSH, AFC (29). However, in an animal model, the daily intake of MTX for 20 days showed a dose- dependent alteration of vaginal cyclicity and hormonal changes toward post-menopausal values. In our study, we did not observe a correlation between the uses of MTX and the AFC or AMH levels although a tendency to lower levels was observed in those women that had been under treatment for more than one year.

Regarding vitamin D levels, the Elecsys system showed comparable levels in both the RA and the control group being not optimal levels for both

groups. However, the Roche Elecsys Vitamin D3 (25 OH) assay has been reported to underestimate vitamin D levels and was removed from the market (30). Therefore, the use of this assay could have influenced the obtained results. Interestingly the vitamin D levels were inadequate for both groups.

Conclusion

In summary, the AMH levels in women with RA were comparable to those found on healthy individuals nevertheless there was no difference in the age of the groups.

However, the RA patients were more likely to have a low AFC. Ovarian reserve and RA were neither influenced by parameters of disease activity nor by the use of MTX. The subfertility reported in RA patients did not appear to be due to a ROR, however further studies should aim to include a higher number of patients and the assessment of the OR by the direct (AFC) and the indirect (AMH) measures. Additionally, the assessment of the OR prior to the use of the MTX for the RA patients should be included to evaluate the impact of the treatment on the OR.

Conflict of Interests

Authors have no conflict of interests.

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