# The ovarian reserve as measured by the anti-Müllerian hormone is not diminished in patients with rheumatoid arthritis compared to the healthy population

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## Abstract

### Objective

*Rheumatoid arthritis (RA) is the most prevalent chronic inflammatory arthritis, affecting 0.5–1% worldwide population and predominates in females. Altered fertility has been reported due to a decrease in ovarian reserve secondary to sustained inflammation. The anti-Müllerian Hormone (AMH) is currently the most reliable biomarker of ovarian reserve. However, few and contradictory studies have been reported to analyse the relationship between fertility in RA female patients and AMH. The aim of present study is to determine the AMH serum concentrations in a long-standing RA patient group and control group. We also sought to determine the correlation between AMH serum levels and disease activity measured by different parameters and the effect of biological DMARDs.*

### Methods

*Serum AMH levels were measured in 60 women with long-standing RA aged 20–50 y.o. and compared to 59 healthy women. AMH was assessed by an electrochemiluminescence immunoassay method (ECLIA, Roche Diagnostics) and a large data set of clinical and molecular data was annotated. Demographic parameters, RA disease activity measured by DAS28 score and inflammatory biomarkers such as ESR, CRP, lymphocyte CD4+, CD8+, NK cells, IL-10 and IL-6 were determined. A comprehensive gynaecological self-administered questionnaire was given. Serum AMH levels were age-correlated. Differences between groups were calculated using Student's t-test or Mann-Whitney U-test for continuous variables and Fisher's exact test for categorical variables. Multivariate analysis was conducted by the partial correlation coefficient. Linear regression analysis was performed to study the effect of different variables on proportional AMH change. p-values <0.005 were considered significant.*

### Results

*The median age was similar in AR and control groups (37.4±6.23 vs. 37.3±6.27 p=0.937). Mean disease duration was 8.37±5.36 years. The number of previous treatments was <3 in 71.7% of patients and ≥3 in 28.3%. Disease activity measured by DAS28 was 2.89± 1.54. The age-adjusted mean serum concentration of AMH was 1.27 ng/ml [IQR 0.42; 2.24] in RA patients and 1.31 ng/ml [IQR 0.46; 3.09] in controls (p=0.608). Neither disease activity (p=0.862), nor current or previous bDMARDs treatments (p=0.871) were associated with AMH levels. However, a negative linear correlation was observed between AMH and IL-10 levels (p=0.033).*

### Conclusion

*Our study shows that ovarian reserve determined by AMH serum levels is not reduced in rheumatoid arthritis patients compared with healthy controls. In our series, AMH levels were not affected by disease activity, however, a significant correlation was observed between AMH and IL-10 levels. These results support the role of cytokines profile in the female reproductive system and will focus further investigations in this critical area, mainly once biological DMARDs have been recommended in RA pregnant patients.*

**Key words**

rheumatoid arthritis, anti-Müllerian hormone, fertility

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*Received on January 26, 2019; accepted in revised form on April 20, 2020.*



*Competing interests: none declared.*

## Introduction

Rheumatoid arthritis (RA) is the most prevalent chronic inflammatory arthritis, affecting 0.5–1% of the population worldwide (1). It predominates in the female gender, with a female: male ratio of 3:1. The reason of gender differences is unknown, although it could be related to the effects of the hormonal environment on immune function (2). A third of these women are diagnosed during their reproductive years, a fact that confers a greater complexity to the follow-up of these patients (3). To date, however, the studies on the impact of RA in fertility have provided heterogeneous results (4, 5).

There is evidence that suggests that RA is associated with a delay in the conception (6). An alteration in fertility has been reported, a concept known as subfertility, in which the time to achieve a pregnancy is greater than 12 months in patients with RA compared to the healthy population (6). The hypotheses that have been postulated about the cause of subfertility in RA are diverse, including the decrease in ovarian reserve secondary to sustained inflammatory activity, adverse effects of treatments or psychosocial aspects such as a decrease in sexual desire or personal choice (7, 8).

The ovarian reserve can be studied by determining the anti-Müllerian hormone (AMH). AMH is a dimeric glycoprotein member of the transforming growth factor β family (TGF-β) that is secreted by the granulosa cells of the antral and preantral ovarian follicles (9). Unlike other fertility markers such as the follicle-stimulating hormone (FSH) or the luteinising hormone (LH), the stability of the AMH over time allows its detection at any time of the menstrual cycle, being the most reliable biomarker of the ovarian reserve (10).

In RA, there have been contradictory results between AMH levels and the disease. Brower *et al*. performed a cross-sectional study analysing AMH levels in 72 patients newly diagnosed with RA and 409 healthy controls and did not find any statistically significant differences between both groups (11). Subsequently Henes *et al*. studied the impact of different rheumatic autoimmune diseases including 30 patients with Behçet's disease, 32 patients with spondyloarthropathy and 33 patients with RA, compared with healthy controls. The results objectified a reduced ovarian reserve with lower AMH levels in all disease groups compared with healthy controls (12). More recently, Eudy *et al* have published a cross-sectional study comparing 75 patients with RA with mean disease duration of 9 years and 75 healthy controls. Despite finding lower AMH levels in RA patients than controls the difference was not statistically significant and patients with other comorbidities apart from RA that can affect ovarian reserve were included (13).

The aim of this study is to determine the serum concentrations of AMH in a cohort of patients with long-standing RA without other comorbidities receiving biological drugs compared to the healthy population.

## Methods

*Patients*

The patients were recruited from the specialised consultation in RA of the the Vall d'Hebron University Hospital. The study was approved by the Clinical Research Ethics Committee and all the patients gave written informed consent. From February 2016 to April 2016, 60 female patients between the ages of 20 and 50 diagnosed with Rheumatoid Arthritis according to the 2010 criteria of the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) were recruited (14).

All patients were receiving treatment with synthetic disease-modifying antirheumatic drugs (DMARDs) and/or biological DMARDs (bDMARDs) for at least 3 months. At the time of inclusion, all patients underwent a complete joint physical examination, applying Disease Activity Score 28 (DAS28), Clinical Disease Activity Index (CDAI), Simplified Disease Activity Index (SDAI) and the Health Assessment Questionnaire (HAQ).

Variables related to the disease and treatments received were collected. A 9 mL blood extraction in heparin tube was performed, and each patient completed a self-administered question-

naire of sociodemographic and gynaecological variables.

*Controls*

Controls were healthy donors from the Blood and Tissue Bank of the Vall d'Hebron University Hospital who agreed to participate in the study and signed the informed consent. Controls were selected to match in age and gender to the patient group. A 9 mL blood extraction in heparin tube was also performed and each control completed the same self-administered questionnaire as the patients.

Exclusion criteria for both groups were pregnancy, history of hypothalamic-pituitary dysfunction, gynaecological tumour, polycystic ovary syndrome, endometriosis, having previously received treatment with cyclophosphamide or other alkylating agents, gynaecological radiotherapy or ovarian surgery. Patients with a concomitant autoimmune disease other than RA were also excluded.

*Measurements*

Disease activity was measured by DAS28. Other variables related to disease activity were also measured, CDAI, SDAI and the HAQ. Laboratory variables included the determination of globular sedimentation rate (ESR), C reactive protein (CRP), rheumatoid factor (RF), anti-citrullinated protein antibody (ACPA), interleukin 6 (IL-6) and interleukin 10 (IL-10). Blood samples were collected in heparin tubes during the first hours of the morning and centrifuged plasma was stored at -80ºC. The AMH was measured by an electrochemiluminescence immunoassay method (ECLIA, Roche Diagnostic, Mannheim, Germany) with standard reference values by age: 20–24 years (3.55–4.33 ng/ml), 25–29 years (3.03–3.87 ng/ml), 30–34 years (2.34–3.55 ng/ml), 35–39 years (1.78–3.24 ng/ml), 40–44 years (0.734–2.13 ng/ml), 45–50 years (0.125–0.498 ng/ml).

*Statistical analysis*

Age is the only well-known prognostic factor for the levels of AMH (9). To improve precision in our analysis we therefore performed a nne-way ANCOVA to test for differences between AMH levels in RA and controls while controlling for age. A square-root transformation was applied to the AMH values to stabilise the variance in the observed data and ensure residual homoscedascity.

We further analysed the association between RA and AMH by adjusting for potential confounding variables. These included: consumption of oral contraceptives (OCs) (yes/no), having had miscarriages (yes/no), number of children, and birthplace (Spain: yes/no).

Associations between clinical variables and AMH levels in RA patients were also tested. These included DAS28, VSG, PCR, IL-10, IL-6, CDAI, SDAI, HAQ and treatment type. The latter variable was the only categorical variable and was defined using 5 levels (Table IV). The same One-way ANCOVA approach for each categorical variable and the analogous linear regression for each continuous variable were used to control for age.

In bivariate analysis, Student's t-test and Mann-Whitney U-test were used for continuous variables and Fisher's exact test for categorical variables. All analyses were performed with R (v. 3.5). The level of significance was set to 0.05.

**Table I.** Characteristics of the study participants.

| | Controls n=59 | Rheumatoid arthritis n=60 | *p*-value |
|---|---|---|---|
| Age, years (SD) | 37.4 (6.23) | 37.3 (6.27) | 0.937 |
| Body mass index (SD) | 25.1 (4.74) | 23.7 (3.59) | 0.071 |
| Birthplace | | | **<0.001** |
| Spain, n (%) | 56 (94.9%) | 41 (68.3%) | |
| North Africa, n (%) | 0 (0%) | 2 (3.33%) | |
| East Europe, n (%) | 0 (0%) | 1 (1.67%) | |
| South America, n (%) | 3 (5.08%) | 16 (26.7%) | |
| Employment status | | | **0.001** |
| Active, n (%) | 52 (88.1%) | 36 (60%) | |
| Unemployed, n (%) | 7 (11.9%) | 24 (40%) | |
| Civil status | | | 0.127 |
| Single, n (%) | 19 (32.2%) | 13 (21.7%) | |
| Married, n (%) | 37 (62.7%) | 38 (63.3%) | |
| Divorced, n (%) | 3 (5.08%) | 9 (15%) | |
| Physical activity | | | 0.162 |
| High activity | 16 (27.1%) | 9 (15%) | |
| Low activity | 43 (72.9%) | 51 (85%) | |
| Daily smoker | | | 0.422 |
| Active smoker | 12 (20.3%) | 17 (28.3%) | |
| Non smoker | 47 (79.7%) | 43 (71.7%) | |
| Oral contraceptives | | | 0.546 |
| Yes, n (%) | 4 (6.78%) | 7 (11.7%) | |
| No, n (%) | 55 (93.2%) | 53 (88.3%) | |
| Period regularity | | | 0.089 |
| Yes, n (%) | 55 (94.8%) | 50 (83.3%) | |
| No, n (%) | 3 (5.17%) | 10 (16.7%) | |
| Period duration, median, IQ | 5 [4;5] | 4 [4;6] | 0.330 |
| Number of children, n (%) | | | 0.834 |
| 0 | 28 (47.5%) | 28 (46.7%) | |
| 1 | 14 (23.7%) | 13 (21.7%) | |
| 2 | 15 (25.4%) | 14 (23.3%) | |
| 3 | 1 (1.69%) | 4 (6.67%) | |
| 4 | 1 (1.69%) | 1 (1.67%) | |
| Reported difficulties to get pregnant | | | 0.06 |
| Yes, n (%) | 4 (6.78%) | 12 (20.3%) | |
| No, n (%) | 55 (93.2%) | 47 (79.7%) | |
| Miscarriages | | | 1.00 |
| Yes, n (%) | 11 (18.6%) | 12 (20.3%) | |
| No, n (%) | 48 (81.4%) | 47 (79.7%) | |

## Results

From February 2016 to April 2016, 60 cases and 59 controls between 20 and 50 years were included. The characteristics of study participants are presented in Table I.

The mean age of the patients and controls was 37.4 (±6.23) years and 37.3 (±6.27) years respectively. 68.3% of the patients were Hispanic compared

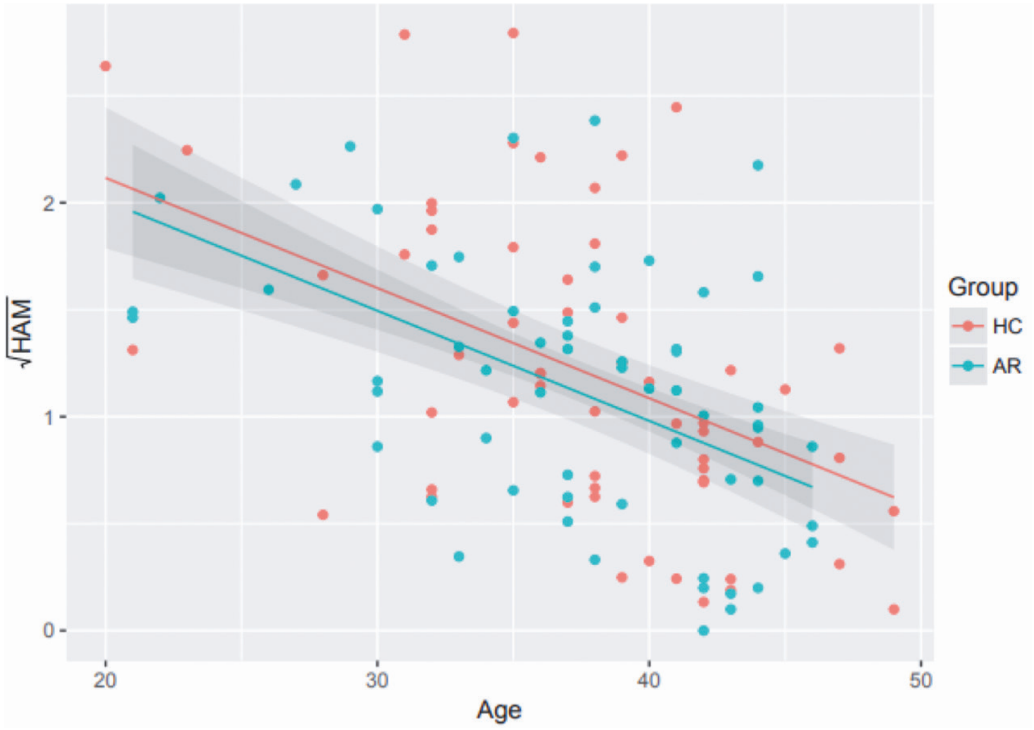


**Fig. 1.** Serum concentrations of AMH in controls 1.22 ng/ml and patients 1.12 ng/ml ($p$=0.32).

**Table II.** AMH serum concentrations between groups.
The three models were hierarchical: Model 1 adjusted for age, Model 2 additionally adjusted for being born in Spain (yes/no), Model 3 additionally adjusted for having had miscarriages (yes/no), having consumed oral AC (yes/no) and number of children. The marginal means were computed at the mean value of the covariates.

| | $\sqrt{AMH}$Marginal mean | | Difference in means (95% CI) | $p$-value |
|---|---|---|---|---|
| | Controls n=59 | Rheumatoid arthritis n=60 | | |
| Model 1 | 1.222 (0.075) | 1.116 (0.074) | -0.106 (-0.315 - 0.103) | 0.32 |
| Model 2 | 1.191 (0.100) | 1.103 (0.079) | -0.088 (-0.311 - 0.136) | 0.44 |
| Model 3 | 1.252 (0.114) | 1.159 (0.095) | -0.092 (-0.318 - 0.133) | 0.42 |

**Table III.** AMH serum concentration associations with other relevant variables.

| | $\sqrt{AMH}$Marginal mean | | Coefficient (95% CI) | *p-value* |
|---|---|---|---|---|
| | No n=108 | Yes n=11 | | |
| Oral AC | 1.227 (0.087) | 1.134 (0.067) | -0.092 (-0.311 - 0.126) | 0.40 |
| Number of childs | - | - | -0.048 (-0.157 - 0.061) | 0.38 |
| Miscarriages | No n=95 | Yes n=23 | | |
| | 1.155 (0.060) | 1.225 (0.121) | 0.070 (-0.198 0.338) | 0.60 |

to 94.9% of the controls, with a greater representation of patients from South America in the group of patients (26.7%) in comparison to the controls (5.08%) ($p$<0.001). There were no statistically significant differences between the groups in terms of employment status, civil status, physical activity or smoking habit. No differences were observed between the use of oral contraceptives, regularity of the menstrual cycle, miscarriages, and reported difficulties to get pregnant or number of children.

The serum concentration of AMH was not significantly different in patients from controls (mean (± SD) concentration in RA patients: 1.12 ng/ml (0.074); mean (±SD) concentration in controls: 1.22 ng/ml (0.075) in the controls group ($p$=0.32) (Fig. 1) (Tables II and III).

No association was found between AMH levels and the disease activity as measured by DAS28 or any of the other activity measurements ($p$>0.5).

No differences were found between AMH concentrations and positivity to RF or ACPA ($p$=0.56 and $p$=0.68, respectively) (Table IV).

While IL6 levels were not associated to AMH, we found that IL10 levels were negatively correlated with the hormone levels ($p$=0.0044) (Fig. 2).

The number of previous treatments received was between 1 and 3 in 71.7% of the patients and greater than 3 treatment lines in 28.3% of the patients. No differences were found between the AMH levels and the number of previous treatments received ($p$=0.87).

Finally, no statistically significant differences were found in the serum concentrations of AMH between the different treatment groups ($p$=0.11) (Table V).

## Discussion

In the last decades research has attempted to elucidate the reason why

**Table IV.** AMH serum concentrations association with AR variables.

| Variable | | Effect CI (95%) | *p*-value |
|---|---|---|---|
| RA years, mean (SD) | 8.4 (5.4) | -0.017 (-0.045, 0.012) | 0.24 |
| FR positive, n (%) | 49 (81.7%) | 1.1 (0.94, 1.3) | 0.56 |
| CCP positive, n (%) | 52 (86.7%) | 1.1 (0.95, 1.3) | 0.68 |
| DAS-28, mean (SD) | 2.9 (1.5) | -0.0056 (-0.098, 0.087) | 0.90 |
| CDAI, median [IQR] | 5 [0;11] | 0.0074 (-0.0084, 0.023) | 0.35 |
| SDAI, median [IQR] | 3.1 [0.18;10] | 0.01 (-0.0053, 0.027) | 0.18 |
| HAQ, median [IQR] | 0.25 [0;1] | 0.08 (-0.15, 0.32) | 0.47 |
| VSG, median [IQR] | 12 [7;28] | -0.0054 (-0.015, 0.0044) | 0.27 |
| PCR, median [IQR] | 0.12 [0.088;0.24] | -0.13 (-0.45, 0.19) | 0.41 |
| IL10, median [IQR] | 0 [0;7.81] | -0.12 (-0.21, -0.041) | 0.0044 |
| IL6, median [IQR] | 21 [9.7;95] | 5.4e-06 (-0.00032, 0.00033) | 0.97 |

fertility is impaired in patients with RA. Whether the increased on time to pregnancy represents a decreased in ovarian reserve is an important question to be addressed. Nowadays the serum AMH is the most useful test to measure the ovarian reserve that can help to predict subfertility.

In our study we did not observe any differences in serum AMH concentrations between patients with RA and healthy controls. To our knowledge, this is the first study that determines this biomarker of ovarian reserve in patients with long-standing RA who have received various treatments throughout the disease and without any other comorbidities rather than the inflammatory arthritis.

Our results are in agreement with those previously reported by Brouwer *et al*. In this study AMH median levels were 1.71 ng/ml in RA patients and 2.82 ng/ml in the controls with no statistically significant difference ($p$=0.254). It has to be taken into account that the patients were newly diagnosed RA and DAS28 at the first visit was moderate (11). Our long-standing RA cohort has had an inflammatory activity maintained for a longer period of time that could have been affected AMH levels. Recently Eudy *et al* have reported similar results as us studying 75 RA patients and 75 controls with mean AMH levels 3.0 ng/ml and 3.9 ng/ml respectively ($p$=0.1). Although RA patients have mean disease duration of 9 years, patients with other important comorbidities such as polycystic ovarian syndrome that can affect AMH levels were included (13). Opposed to the previous results, Henes *et al*. studied a young RA cohort with mean age of 26 years who had significantly reduced AMH levels compared with controls (1.8 ng/ml and 2.4 ng/ml, respectively) ($p$=0.007) (12). However, the relatively small number of patients and unknown cytotoxic previous treatments in RA patients could explain these results.

It has been hypothesised that fertility impairment in women with autoimmune diseases like Sjögren's syndrome or SLE could be related to the presence of specific autoantibodies that cause autoimmune oophoritis. Vega *et al*. found that AMH levels decline with the presence of antiphospholipid antibodies (15). In this regard, ACPA antibodies and Rheumatoid Factor can be found years before disease onset and are related with a severe prognosis. However, equally to the previous studies, we did not find any association with lower concentrations of AMH and the presence of RF or ACPA in the RA patients (11-13).

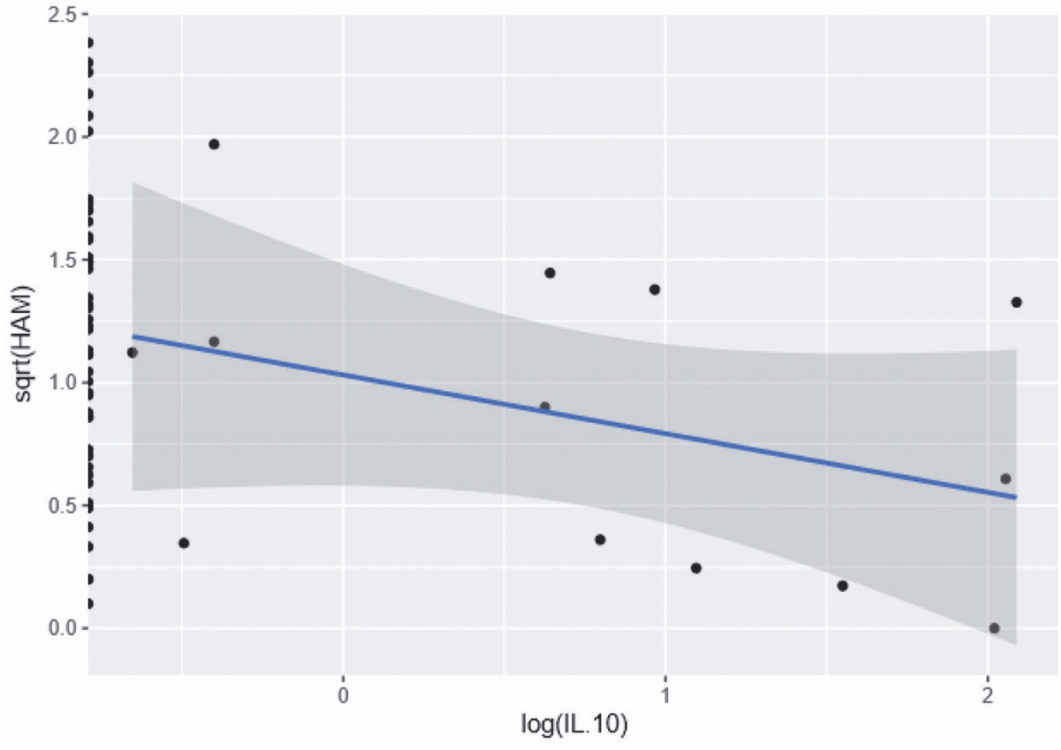


**Fig. 2.** Negative correlation between AMH levels and IL10.

**Table V.** AMH serum concentrations in each treatment group. Group 1: DMARDs monotherapy; Group 2: bDMARD anti-TNF monotherapy; Group 3: bDMARD non anti-TNF monotherapy; Group 4: DMARDs in combination with bDMARD anti-TNF; Group 5: DMARDs in combination with bDMARD non anti-TNF.

| Treatment group | Number (%) | Mean | CI | *p*-value |
|---|---|---|---|---|
| Group 1 | 12 (20%) | 1.20 | (0.92 – 1.50) | 0.11 |
| Group 2 | 20 (33.3%) | 1.10 | (0.83 – 1.30) | |
| Group 3 | 6 (10%) | 1.60 | (1 – 1.20) | |
| Group 4 | 13 (21.7%) | 0.88 | (0.58 – 1.20) | |
| Group 5 | 9 (15%) | 1.10 | (0.78 – 1.50) | |

Regarding RA treatment, it ha been suggested that disease-modifying drugs such as methotrexate are involved in the reduction of the ovarian reserve. In murine models, the daily administration of methotrexate disrupts the female sexual hormone cycle in a dose-dependent manner, even at low doses, leading to hormonal values close to menopause (16). In our cohort, Group 1 received methotrexate in monotherapy and no diminished AMH levels were found regarding other treatment groups. Similarly, Brouwer *et al*. did not find any differences in the concentrations of AMH in their RA patients after 6 months of methotrexate therapy (11). Still on the subject of RA treatment, it is remarkable the change in the last decade in RA prognosis since the appearance of bDMARDs and small molecules like JAK inhibitors.

In our study, we did not find significant differences in serum AMH concentrations comparing all the treatment groups (Table V). To our knowledge, there are no previous studies analysing the alteration of the ovarian reserve in RA patients treated with non-anti-TNF biological drugs like abatacept, tocilizumab or tofacitinib.

The British Society for Rheumatology (BSR) in 2016 and the European League Against Rheumatism (EULAR) task force in 2017 have established statements on the compatibility of antirheumatic drugs during pregnancy and lactation (17, 18). Compatibility with pregnancy and lactation was found for antimalarials, sulfasalazine, azathioprine, ciclosporin, tacrolimus, colchicine, intravenous immunoglobulin and glucocorticoids. NSAIDs should be restricted to the first and second trimesters. Among biologics tumour necrosis factor inhibitors are the best studied and appear safe with first and second trimester use. Data regarding other antirheumatic biologics or small molecules are scarce and further studies are needed to rule the potential impact on pregnancy and embryo/foetal development. Despite the progress made regarding the introduction and maintenance of biological DMARDs in the preconception and during pregnancy, there is still controversy in their use by either rheumatologist or obstetricians. Fayad and colleagues have recently published the results form a questionnaire completed by rheumatologist and obstetricians practicing throughout Lebanon, studying practice patterns in the management of women with rheumatic diseases. The study evidences disagreement regarding the beliefs of drug compatibility, finding that over 30% to 60% of rheumatologists and obstetricians thought that NSAIDs and anti-TNF alpha are not compatible with pregnancy and breastfeeding (19).

Regarding disease duration, the long evolution time of the disease (8.37 years) was not associated with lower AMH levels. Eudy *et al*. similarity reported a cohort with long-standing RA and despite finding lowers AMH levels in patients the difference was not statistically significant (13). The activity of the disease in our cohort was low, with a mean DAS28 of 2.89, without objectifying a correlation with AMH. Brower *et al* did not find any significant correlation between AMH and DAS28 in a higher disease activity cohort (11).

We found no association with other parameters of disease activity such as acute phase reactants or composite indices of CDAI, SDAI or HAQ. When testing the systemic levels of proinflammatory cytokine IL6 and anti-inflammatory IL10, we found a significant association between the latter and AMH levels ($p$=0.0044).

Cytokines have shown to be important for the reproductive process and their function has been studied extensively during controlled ovarian stimulation (COS) in *in vitro* fertilisation (20). Studies carried out in COS have shown that the implantation rate can be negatively affected by the presence of IL10 in serum (21). Despite our results, these findings have to be interpreted with caution and future studies should be done with a larger cohort of patients.

In this study we have analysed diverse gynaecological and obstetric variables in relation to AMH levels in patients and controls. We did not find any differences between in the number of children or number of abortions in both groups. On the other hand, we found that in both groups the serum concentrations of AMH were lower in the users of ACOs but not statistically significant. The ACOs have an influence on the ovarian volume, especially in the antral follicles constituted by a greater number of granulosa cells secreting AMH. Therefore, concentrations of AMH will be reduced in the users of ACOs, an aspect to be taken into account whenever we evaluate the parameters of ovarian reserve in these patients (22).

The results presented here have several limitations. First the sample size, although bigger than previous studies, is relatively modest. In addition, there are factors that influence the ovarian reserve that have not been examined in this study like the use of concomitant therapies like NSAIDs or GC.

In conclusion, the present study adds further evidence that the ovarian reserve is not affected by RA compared to controls. Also, we did not find evidence that disease activity, previous and current treatments influence the ovarian function, as captured by the AMH. These results can help the rheumatologist to advice patients with RA on their family planning. Additional studies are required to determine the course of ovarian reserve abnormalities and develop biomarkers that allow early detection of ovarian reserve failure.

## Acknowledgements

We wish to thank the nurses from the Blood and Tissue Bank of the Vall d'Hebron Barcelona Hospital Campus who helped with recruiting healthy donor samples.

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