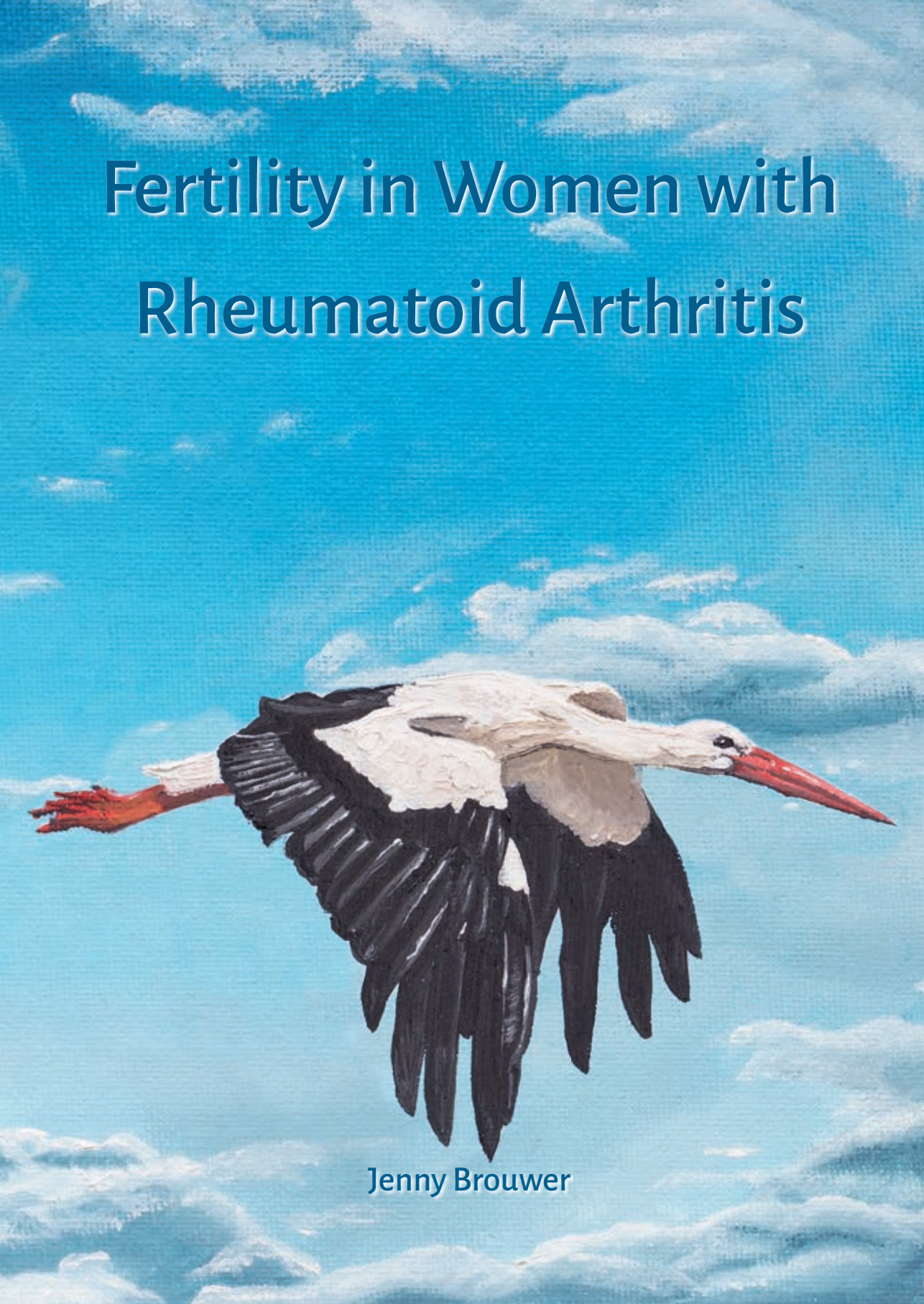
Fertility in Women with Rheumatoid Arthritis



Jenny Brouwer

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Vruchtbaarheid van vrouwen met reumatoïde artritis

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Colofon

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Chapter 1

General introduction

Rheumatoid arthritis is a common disease which seems to be interlinked with impaired fertility. How exactly this disease might affect fertility is largely unknown. This introduction will provide an overview of the possible interaction between rheumatoid arthritis and fertility. Before doing so we will provide some basic understanding of rheumatoid arthritis in general. Next, a description is given of ovarian function and its regulators. Furthermore, the available literature on fertility in rheumatoid arthritis will be summarized. The latter will be followed by a description of the design of the PARA study, which has been the fundament for the main part of this thesis. Finally, the objectives of this thesis will be described as well as its outline.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic systemic inflammatory auto-immune disease. With a prevalence of 0.5 – 1.0 % of adults in Western countries, it is one of the most common auto-immune rheumatic diseases.[1] Typically, RA occurs more often in females than in males.[2,3] The incidence of RA increases with age.[4] Over all ages, and especially in adults younger than 45 years, more women than men are affected.[2]
Over time, RA can lead to permanent joint damage[5], but extra-articular manifestations are also common.[4] In patients with RA, mortality rates are increased, amongst others through an increased risk from cardiovascular, infectious, hematologic, gastrointestinal , and respiratory comorbidities and complications.[6]

Serology

Autoantibodies are often present in RA. The presence of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) can precede a clinical diagnosis of RA by years.[7,8] RF was the first known antibody associated with RA, and has been part of past and future classification criteria for RA.[5,9,10] ACPAs have been discovered more recently, and their presence is highly specific for the development of RA.[10] Furthermore, ACPAs are more discriminative than RF in distinguishing RA from other arthritic diseases.[11] Moreover, ACPA positive patients appear to have a different form of RA than ACPA negative patients, with ACPA positive patients having a higher chance of radiological joint damage, more extra-articular manifestations, and a different response to anti-rheumatic therapy, often requiring a more intensive treatment strategy.[10]

Diagnosis

For decades, patients were diagnosed with RA when the disease had already reached a destructive state and had caused permanent joint damage. For study purposes,

several sets of classification criteria have been developed over the years. Of these, the 1987 the American College of Rheumatology (ACR) criteria have been applied for many studies worldwide.[9] The 1987 ACR criteria for classification of RA include: morning stiffness, the number of affected joints, the location of the affected joint areas, symmetry of arthritis, the presence of rheumatoid nodules, the presence of serum RF, and radiographic changes. When 4 out of 7 criteria are present for six or more weeks, a patient is considered having certain RA.[9]

In 2010, a new set of classification criteria was developed to allow for early recognition of possible RA, and consequently for early start of anti-rheumatic treatment: the 2010 ACR/European League Against Rheumatism (EULAR) classification criteria.[5] When applying these criteria, points are assigned to the number of large or small joints involved, the presence of low-positive or high-positive RF or ACPA, abnormal C-reactive protein (CRP) levels or erythrocyte sedimentation rate (ESR), and the duration of symptoms ≥6 weeks. When a patient has 6 or more points, a diagnosis of definite RA can be made.[5]

Disease activity score

To compare disease course between patient groups and monitor patients' responses to anti-rheumatic therapy, RA disease activity can be measured using the Disease Activity Score (DAS) with a 44 joint count, or the modified DAS28 using a 28 joint count.[12] Aside from the tender and swollen joint count, the DAS also includes the ESR or the serum CRP level, and optionally a visual analogue scale for global health (GH). By selecting the items included in the DAS, the score can be adjusted to specific situations, such as pregnancy. Overall changes in ESR and GH scores in pregnant women may influence the DAS. It has been shown, that the DAS28-CRP without GH component, is the least affected by pregnancy itself.[13] The DAS28 ranges from 0 to 10, indicating how active the RA is at that moment. Remission criteria have also been developed, with patients with a DAS28 below 2.6 being considered in remission.[14]

Anti-rheumatic treatment

To prevent or limit long-term damage due to RA, the current EULAR guideline (2010) recommends the start of anti-rheumatic drugs as soon as a patient is diagnosed, or when a diagnosis of RA is suspected. The guideline describes a treat-to-target regimen with synthetic disease modifying anti-rheumatic drugs (DMARDs) as first step treatment after the diagnosis of RA.[15] Anti-rheumatic therapy should aim at disease

remission or alternatively a state of low disease activity. Methotrexate (MTX), a folic acid antagonist, is the treatment of first choice. Other synthetic DMARDs include sulfasalazine, leflunomide, and antimalarial drugs such as hydroxychloroquine. Combination therapy with several synthetic DMARDs has been proven to be more effective than monotherapy.[16,17] Since in general DMARD therapy starts to be effective after 6 to 12 weeks, the fast-acting glucocorticoids can be added as a bridging therapy to the DMARDs for a short period of time to enhance the anti-rheumatic effect.[15] When therapy with synthetic DMARDs does not result in low disease activity or remission, biologic DMARDs such as tumor necrosis factor (TNF) inhibitors are the next treatment option.[15] When a patient achieves persistent remission, anti-rheumatic drugs can be tapered with caution, one by one, always aiming at sustained remission for each individual patient.[18]

Aside from synthetic or biologic DMARDs, RA patients often use non-steroidal antiinflammatory drugs to manage pain and stiffness. However, the use of NSAIDs does not prevent long term damage in RA.[1]

Anti-rheumatic treatment during the preconception period

When a woman with RA wishes to conceive, the most common first-step treatment, the folic acid antagonist MTX, is contra-indicated because of teratogenicity.[19] Because of insufficient evidence of the safety of numerous other anti-rheumatic drugs, such as leflunomide, abatacept, rituximab, tocilizumab, ustekinumab and anakinra, expert advice has been to discontinue these agents.[20,21] In a subgroup of patients, anti-rheumatic treatment is stopped completely during the preconception period or in early pregnancy.[22] However, it is advised not to stop all anti-rheumatic treatment completely, because active disease in the mother can lead to a less favourable outcome of pregnancy. A higher disease activity during pregnancy has been associated with a lower birth weight, and an increased risk for delivery through caesarean section.[23] Recently, an EULAR task force on anti-rheumatic drugs before, during and after pregnancy has reported that active disease can be treated effectively with reasonable safety for the foetus or newborn during pregnancy and lactation.[20]

In daily practice, many rheumatologists have been reluctant of prescribing medication during pregnancy, due to possible teratogenicity or adverse effects during pregnancy.[21] Therefore, over the last decade pregnant women with RA were mainly treated with steroids, sulfasalazine, and hydroxychloroquine, or did not receive any anti-rheumatic treatment despite active disease during the preconception period

and in pregnancy. Often, NSAIDs were added for pain management.[21] A minority of pregnant RA patients received biologic DMARDs.[22,24] Over the past years, more data on the use of the biologic DMARDs has become available. Since TNF inhibitors appear to be safe before and during pregnancy, at least until the end of the second trimester[25-28], their use among women with RA trying to conceive is increasing nowadays.[20]

Ovarian function and ovarian ageing

Female fertility decreases with increasing age. Generally, women above 30 years of age are considered to have gradually decreasing fertility over time, with an acceleration in declining fertility after the age of 37 years. On average female fertility is considered to come to an end around an age of 40 years.[29] However, this varies considerably between women since there are women who lose their fertility as early as 30 years of age, or as late as 45 years of age. This decrease in fertility with increasing age is attributed to a decreasing ovarian function through the reduction in the number of ovarian primordial follicles over time and a concomitant decrease in oocyte quality.[30,31] In the female foetus, mitosis of germ cells results in approximately 7,000,000 oocytes in the developing ovaries.[32] Once they have entered meiosis, the number of oocytes ceases to increase.[32] During early folliculogenesis, when somatic cells surround the oocytes to form primordial follicles, the majority of germ cells are eliminated. As a result, a number of 700,000 to 1,000,000 oocytes are present in the ovaries at birth.[33,34] During childhood, the number of oocytes further decreases, and at menarche, the primordial follicle pool consists of approximately 400,000 oocytes.[33]

During the reproductive period, every menstrual cycle a number of around 800-1000 primordial follicles will grow and leave the primordial pool. Rising and subsequent decreasing levels of follicle stimulating hormone (FSH) will cause one follicle to become dominant, which can be fertilized after ovulation. The other follicles that left the primordial follicle pool will become atretic and are lost.[27] This cyclic recruitment and subsequent follicle atresia cause a continuous decline of the primordial follicle pool. (Figure 1) When the primordial follicle pool is nearly exhausted and only contains approximately 1,000 follicles, no more cyclic follicle growth occurs and anovulation sets in. As a definite hallmark a woman's menstrual periods will stop and hence menopause occurs[33]. On average, menopause is reached at an age of 50-51 years, with a broad range spanning 20 years and ranging from 40-60 years.[31] However, in populations not interfering with natural infertility the age at which the last child is born is on average around an age of 40-41 years. Hence, fertility and also fecundity

ends approximately 10 years before menopause occurs.[31] It is therefore that the age at which fertility ends has a similar broad range as the occurrence of menopause.

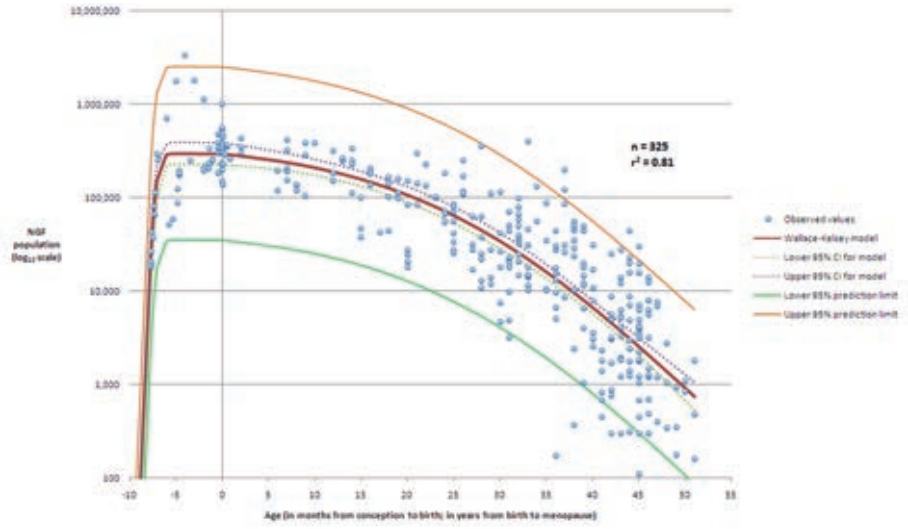

Figure 1 – Wallace-Kelsey model describing the nongrowing follicle populations (NGF) from conception to menopause, and the 95% confidence intervals and 95% prediction limits for the model. The predicted average age at menopause with this model is 49.6 years (95%PI 38.7–60.0 years). (Duplicated with permission from the author, source: Wallace, PLoS One 2010.[35]

Anti-Müllerian hormone as a measure of ovarian function

The size of the ovarian follicle pool can be estimated by measuring serum levels of anti-Müllerian hormone (AMH). AMH is a member of the transforming growth factor β family, and is well known for its role in the regression of the Müllerian ducts in the male foetus.[36] In women, AMH is specifically produced by the granulosa cells of the small growing ovarian follicles.[33,37] AMH levels are first detectable in the female foetus at 36 weeks of gestation, when ovarian primordial follicles start developing into primary follicles.[38] AMH expression is highest in secondary, preantral and small antral follicles (≤4 mm), until it disappears in larger antral follicles (4-8 mm).[37]

AMH has a function in the regulation of follicle recruitment. In AMH knock-out mice, there is an increased rate of follicle recruitment, which leads to premature depletion of the ovarian follicle pool and consequently anovulation.[39] Furthermore, AMH affects the sensitivity of follicles to follicle stimulating hormone (FSH) from the pituitary gland. In the absence of AMH, the individual threshold of a follicle for FSH seems to be lower.[39]

In women, AMH levels are highest during early adulthood. After that, just like the number of ovarian follicles, AMH levels decline over age, until serum AMH is undetectable around menopause.[18]
Currently, serum AMH levels are the most reliable predictor for the age at which a woman will enter menopause.[40] Where other hormonal markers for ovarian function, such as follicle stimulating hormone (FSH), might vary throughout the menstrual cycle, serum AMH concentrations are fairly stable throughout the menstrual cycle phases.[41] Over the years, a variety of AMH assays have been developed, but large comparison studies of the different assays are lacking.[42,43] More recently ultra-sensitive assays have been introduced, which are able to measure serum AMH levels below the previous limits of detection.[44]

In pregnant women, lower serum AMH levels have been reported, with the lowest levels measured during the third trimester of pregnancy.[45-48] The effect of oral contraceptives use on AMH levels has been studied, but results are inconclusive, with several studies reporting no effect of hormonal contraceptives on serum AMH levels[49-51], and others finding decreased AMH levels in users of hormonal contraceptives.[52-54]
Regarding fertility, AMH levels have been reported to add to the prediction of live birth in assisted reproductive technology cycles[55-59], and low serum AMH levels have been reported to be associated with a reduced chance of natural conception[60], although results are not very consistent.[61]

Subfertility

In the majority of the general population, a pregnancy is achieved within one year of actively trying to conceive. However, for 10 to 15 percent of the couples having regular unprotected intercourse aiming at achieving a pregnancy, this goal is not met within 12 months and they are considered subfertile.[62]

Several factors can compromise a couple's fertility. In the male partner, subfertility is often caused by a reduced sperm count (oligospermia), or absence of spermatozoa in the ejaculate (azoospermia). Causes can be pretesticular, testicular, and post-testicular. Pretesticular disorders are mainly endocrine disorders.[63,64] Testicular disorders, also known as primary testicular dysfunction, include congenital and genetic disorders, gonadotoxins including certain medication, and damage resulting from infection or trauma. Posttesticular causes include ejaculatory dysfunction, such as retrograde ejaculation, and obstructions of the reproductive tract.

The main causes of subfertility in women are anovulation, tubal occlusion, and endometriosis.[65-67] Anovulation can have a central cause (hypogonadotropic hypogonadism), or an ovarian cause (such as ovarian failure, or polycystic ovary syndrome). Tubal occlusion can be bilateral or unilateral.
In up to 30% of subfertile couples, no obvious cause is detected and hence they are referred to as couples with unexplained subfertility.[65-67]

Fertility in rheumatoid arthritis

In the past sixty years, numerous solitary reports on reproduction in women with RA have been published. The two oldest reports both describe a smaller family size in women with RA[68,69], which has been confirmed over time by several studies[70-74]. Nulliparity is more common in women with RA compared to controls[70,75] and a single study has related this to the presence of RF[69]. When looking at the time to pregnancy (TTP), women with RA had a longer TTP than controls for the first pregnancy and similarly for consecutive pregnancies either after or before the onset of RA.[70] Already before disease onset, a TTP longer than 12 months was found in 42% of RA patients.[76] In a group of pregnant RA patients, 25 percent spent more than 12 months before they conceived.[77] Moreover, more women with RA received fertility treatments compared to healthy women without RA.[78]
It has been reported that one in five women with RA consider their disease in their childbearing decisions. These women had significantly fewer pregnancies and fewer children than women who did not consider RA in childbearing decisions.[72]

Miscarriages in RA

The aim of reproduction is a live born baby after an uneventful pregnancy. Miscarriage, being early pregnancy loss before 16 weeks of gestation, occurs in 10-20% of pregnant women, with a the miscarriage rate increasing with advancing maternal age.[79]
Reports on miscarriages in RA patients are scant and have produced contradictory results. An increased miscarriage rate has been reported by several authors[70,78,80], whereas other studies have found no increase in miscarriage rates in RA patients.[69,81-83] When RA patients are exposed to MTX after conception, which is often the case in unplanned pregnancies in this patient group, the incidence of miscarriages is increased.[19]

Menopause in RA

Compared to healthy control women, menopause occurs slightly earlier in women with RA.[69,70] Even in women with postmenopausal onset of the disease, the mean age at menopause was significantly lower compared to matched controls.[69] However, not all studies have confirmed the younger age at menopause.[75]

Studying reproduction in RA – the PARA cohort

This thesis focuses mainly on patients from the Pregnancy-induced Amelioration of Rheumatoid Arthritis (PARA) study. The PARA study aimed to study the changes in the disease course of RA during pregnancy and the postpartum period.[22] The PARA cohort was a nationwide observational prospective cohort, for which eligible patients from the Netherlands were recruited by their attending rheumatologists from May 2002 until November 2008. Patients were eligible when they had a diagnosis of RA according to the 1987 revised ACR criteria[9] and were trying to conceive or were already pregnant. Patients had to have a good understanding of the Dutch language. During the study, patients received usual medical care from their attending rheumatologist. Teratogenic medications, such as MTX, had to be stopped at least 3 months before trying to conceive.

Members of the PARA research team visited the patients at their homes during the preconception period (if possible), once during each trimester of pregnancy, and at six, twelve and 26 weeks postpartum. At each visit, questionnaires on health, medication use, and obstetric information were filled out. Blood and urine samples were collected at each assessment, and the RA disease activity was measured using a standardized swollen and tender joint count for 28 joints, which was combined with the serum C-reactive protein (CRP) levels (DAS28-CRP).[13]

During the years of observation within the PARA cohort, it appeared that many patients had problems achieving a pregnancy. It often took patients longer to conceive, and a considerable number of patients needed artificial reproductive treatments to achieve pregnancy.[23]

Objectives and outline of this thesis

This thesis aims to give an overview of fertility in women with RA, and to look further into the association of fertility with RA disease characteristics and the use of anti-rheumatic drugs. Therefore, this thesis has the following objectives:

(I) to study the time to pregnancy and the occurrence of subfertility in women with RA, and their associations with clinical aspects of RA,
(II) to look into the occurrence of miscarriages and its association with RA disease characteristics,
(III) to study the ovarian function in women with RA, and its role in the TTP in female RA patients trying to conceive, and
(IV) to investigate the long-term decrease of ovarian function over time in women with RA.

The first part of this thesis, chapters 2, 3 and 4, will focus on fertility and reproductive outcome in women with RA. Chapters 5, 6 and 7 will assess ovarian function in RA patients. Next, chapter 8 will reflect on the results, discussing how these impact our current understanding of fertility in RA, what their implications for clinical practice are, and giving suggestions for future research. Finally, chapters 9 and 10 will summarize the main findings of the different studies in this thesis.

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Chapter 2

Fertility in women with rheumatoid arthritis: influence of disease activity and medication

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ABSTRACT

Objectives: Many female rheumatoid arthritis (RA) patients attempting to conceive have a time to pregnancy (TTP) of >12 months. During this period RA often cannot be treated optimally. We sought to identify clinical factors associated with prolonged TTP in female RA patients.

Methods: In a nationwide prospective cohort study on pregnancy in RA patients (PARA study), women were included preconceptionally or during first trimester. Cox regression analysis was used to study the association of disease characteristics and medication use with TTP.

Results: TTP exceeded 12 months in 42% of 245 patients. Longer TTP was related to age, nulliparity, disease activity (DAS28), and preconception use of non-steroidal anti-inflammatory drugs (NSAIDs) and prednisone. These variables were independently associated with TTP, with HRs for occurrence of pregnancy of 0.96 (95% CI 0.92 to 1.00) per year of age, 0.52 (0.38 to 0.70) for nulliparity, 0.81 (0.71 to 0.93) per point increase in DAS28, 0.66 (0.46 to 0.94) for NSAIDs and 0.61 (0.45 to 0.83) for prednisone use. The impact of prednisone use was dose dependent, with significantly longer TTP when daily dose was >7.5 mg. Smoking, disease duration, rheumatoid factor, anti-citrullinated protein antibodies, past methotrexate use, and preconception sulfasalazine use did not prolong TTP.

Conclusions: TTP in RA is longer if patients are older or nulliparous, have higher disease activity, use NSAIDs or use prednisone >7.5 mg daily. Preconception treatment strategies should aim at maximum suppression of disease activity, taking account of possible negative effects of NSAIDs use and higher prednisone doses.

INTRODUCTION

Conceiving a child is a major life event and most adults try to have a child during their reproductive life span. In women with inflammatory rheumatic disease, however, it seems to be more difficult to achieve parenthood.[1]

Rheumatoid arthritis (RA) is one of the most prominent inflammatory diseases affecting women of child-bearing age. Nearly one-third of female RA patients diagnosed before completion of child-bearing experience fertility problems.[2] They have a prolonged time to pregnancy (TTP), the time between the start of actively trying to conceive and actually becoming pregnant.[3] Pregnant RA patients are more likely to have had fertility treatment than pregnant controls.[3,4] Women with RA have fewer children than women without RA,[5-9] and more often fail to conceive at all.[7] Hence fertility as well as fecundity seems to be compromised in women with RA.

Subfertility in RA has been studied only in retrospective studies or comparisons of registries.[2,3,5-9] None of these studies have extensively examined the causes underlying the higher subfertility in RA, which may include disease activity, anti-rheumatic medication and immunological factors.

To identify clinical factors associated with a higher rate of subfertility in women with RA, we studied the TTP in consecutive RA patients who participated in a large prospective cohort study in the Netherlands on Pregnancy-induced Amelioration of Rheumatoid Arthritis (the PARA study).[10]

METHODS

Patients

Patients were drawn from the PARA study, an observational nationwide prospective cohort study on pregnancy in RA.[10] The study was approved by the Erasmus MC medical ethics review board.

From May 2002 until August 2008, rheumatologists in the Netherlands recruited RA patients defined according to the 1987 revised criteria of the American College of Rheumatology (ACR).[11] To be eligible for the PARA study, patients should be actively

trying to conceive, or already pregnant. Teratogenic drugs, such as methotrexate (MTX), should have been stopped for at least 3 months.
Only patients included preconceptionally or during the first trimester of pregnancy were eligible for the current analysis. If a patient participated twice or more, only the first study episode was included.

Data collection

Patients were preferably visited before conception, during each trimester, and three times after delivery. Patients who did not conceive within 1 year after the first visit were visited again 1 year later. At each visit, patients filled out questionnaires, were interviewed by a research team member, and provided details on variables possibly influencing fertility such as age, parity and smoking habits. Disease activity was measured and serum samples were stored at -80°C. Using a structured questionnaire, the researcher recorded the use, frequencies and dosages of anti-rheumatic medication. At the first visit, the researcher recorded the date the patient first began actively trying to conceive.

Patients who were already pregnant during the first visit were asked when their last menstrual period had started. We calculated the TTP as the time elapsed between the first attempt to conceive and the first day of the last menstrual period before pregnancy. If the date of the last menstrual period was not known by the patient, this date was calculated by subtracting 280 days (40 weeks) from the due date based on sonographic examination during early pregnancy. In several cases the couple started to try for pregnancy after the start of the last menstrual period and succeeded within that first month. To avoid negative values for the TTP, we calculated a fictitious date for the pregnancy test for all patients by adding 28 days to the start of the last menstrual period. These dates were used only in the univariate survival analyses and the Cox regression analyses.

Measurements

Disease activity scores were calculated using the 28-joint Disease Activity Score (DAS28) with three variables based on the C-reactive protein (CRP) level (mg/L).[12] We categorised the disease activity scores according to the recommendations of the European League against Rheumatism (EULAR): in remission (DAS28≤2.6), low disease activity (2.6<DAS28≤3.2), intermediate disease activity (3.2<DAS28≤5.1) and high disease activity (DAS28>5.1).[13]

Rheumatoid factors (RF) were measured by commercial ELISA (HYCOR Biomedical, Garden Grove, California, USA) or by the EliA RF IgM method on the ImmunoCAP 250 (Phadia, Uppsala, Sweden). For RF, the level above which only 5% of healthy controls were tested as positive was defined as positive. The presence of anti-citrullinated protein antibodies (ACPA) was tested by fluoroenzyme immunoassay using EliA CCP on the ImmunoCAP 250. A level >10 U/mL was considered positive.[14]

Statistics

Values are given as mean±SD, number (percentage), or median (IQR). We calculated inter-group differences using the Student t test or Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. Differences between different time points were calculated by a paired t test or the Wilcoxon signed-ranks test.

Differences in TTP per categorised variable were studied using Kaplan-Meier curves. The significance of differences between curves was tested using the log-rank test. A multivariable analysis was performed by Cox regression analysis including age, nulliparity, smoking, disease duration, RF, ACPA, DAS28, non-steroidal anti-inflammatory drug (NSAID) use, prednisone use, sulfasalazine use and previous MTX use. If the patient had not become pregnant at the last time of contact, the TTP was considered censored at the date of the last visit or contact.

A two-sided p-value of <0.05 was considered significant. The statistical package Stata/SE V.12.0 for Windows (StataCorp LP, College Station, TX, USA) was used.

RESULTS

Patients

Of 475 patients recruited from May 2002 to August 2008, 369 were enrolled in the PARA study, and 245 of these were available for the present analyses (figure 1). There were no statistical differences in general characteristics between included and excluded subjects.

Study population details are shown in table 1. During the study period, 205 women (84%) conceived, 64 of whom (31%) had a TTP longer than 12 months. These 64 women together with the 40 women who did not become pregnant during follow-up, formed the subfertile group in this cohort (subfertility 42%). In the women who got pregnant, the

median TTP was 0.50 year (IQR 0.19–1.28). Pregnancy resulted in a live born baby in 178 women (87%), 26 women (13%) miscarried and there was one intra-uterine fetal death. Thirty-five pregnant women had had fertility treatment. These women did not differ significantly from the other subjects. No data on fertility assessments were available. Since only a few patients used cyclo-oxigenase-2 (COX-2) inhibitors, traditional NSAIDs and COX-2 inhibitors were regarded as one group for analyses.

In 61 women (25%) who were not assessed preconceptionally, only the first trimester DAS28 was available. In women who had been visited both preconceptionally and during pregnancy (n=109), a paired t test showed no significant difference between the preconception DAS28 (3.57±1.1) and the first trimester DAS28 (3.56±1.2; p=0.93). The first trimester DAS28 in these 109 women did not differ from the first trimester DAS28 in women who had not been assessed preconceptionally (3.53±1.1; p=0.86)., The first trimester DAS28 was used for further analyses if the preconception DAS28 was missing.

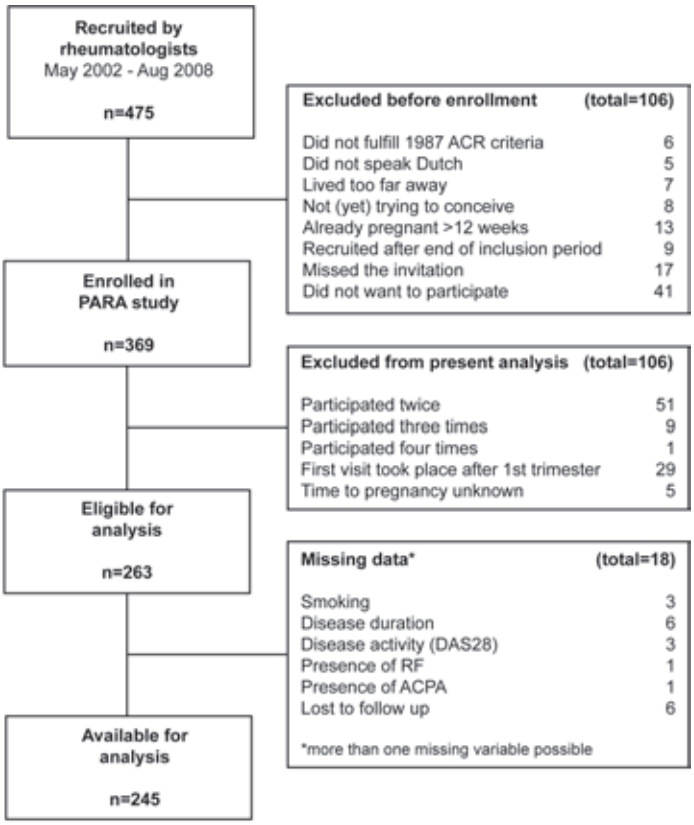

Figure 1 – Flow chart showing the number of patients in the Pregnancy-induced Amelioration of Rheumatoid Arthritis (PARA) study who were available for the current analysis. ACPA, anti-citrullinated peptide antibodies; ACR, American College of Rheumatology; DAS28, 28-joint Disease Activity Score; RF, rheumatoid factor.

Table 1 – Descriptive statistics of the study population

Variable		Value*
Age, years		31.3±3.9
Nulliparity, n (%)		143 (58%)
Smoking, n (%)		34 (14%)
Duration of RA, years		3.6 (1.3–8.4)
RF positive, n (%)		180 (73%)
ACPA positive, n (%)		161 (66%)
Erosions present, n (%)		122 (50%)
MTX in the past, n (%)		161 (66%)
Biologicals in the past†, n (%)		37 (15%)
Disease activity (DAS28)		3.7±1.2
Preconceptional medication, n (%)		
	None	88 (36%)
	Sulfasalazine	79 (32%)
	Prednisone	85 (35%)
	Hydroxychloroquine	15 (6%)
	Non-selective NSAIDs	48 (20%)
	COX-2 inhibitors	12 (5%)
	Biologicals†	10 (4%)
	Other‡	20 (8%)

*Values are given as mean±SD, n (%) or median (25^{th}–75^{th} percentile).

†Biologicals: etanercept, adalimumab, infliximab, anakinra.
‡Other medications including gold, azathioprine and leflunomide.
ACPA, anti-citrullinated peptide antibodies; COX-2, cyclo-oxygenase-2; DAS28, 28-joint Disease Activity Score; MTX, methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; RA, rheumatoid arthritis; RF, rheumatoid factor.

Overall, 67% of women in the high disease activity group (DAS28>5.1), 43% of women in the intermediate disease activity group (3.2<DAS28≤5.1), 37% of women in the low disease activity group (2.6<DAS28≤3.2) and 30% of women in remission (DAS28<2.6) were subfertile (figure 2A).

In the subfertile group, age and DAS28 were significantly higher than in the fertile group (table 2). Subfertile patients were more frequently ACPA positive and used NSAIDs and prednisone more often.

The women who did not become pregnant at all (n=40) had a significantly higher DAS28 (4.14±1.3) than those who became pregnant (3.61±1.1; p=0.008).

RF positivity was more common in women who did not conceive (90%) than in those who did (70%; p=0.01). ACPA was positive in 80% of women who did not conceive compared to 63% of women who did get pregnant (p=0.05). Disease duration did not differ significantly: it was 4.4 (1.7–7.8) years in non-pregnant women and 3.4 (1.2–8.5) years in women who did get pregnant (p=0.76).

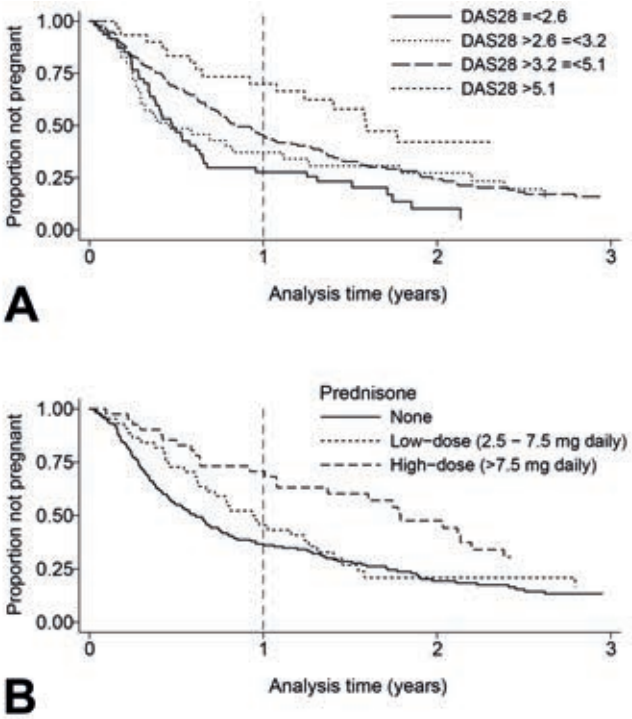

Figure 2 – Survival curves showing the time to pregnancy (TTP) in rheumatoid arthritis (RA) patients with (A) various levels of disease activity and (B) different prednisone dosages. When the TTP exceeded 1 year, patients were considered subfertile. If women had not become pregnant at the last time of contact, the TTP was considered censored at the date of the last visit. (A) Patients with high disease activity (DAS28>5.1) had a longer TTP than patients in the other groups (3.2<DAS28≤5.1, p=0.03; 2.6<DAS28≤3.2, p=0.04; DAS28<2.6, p=<0.001). Patients with intermediate disease activity (3.2<DAS28≤5.1) had a longer TTP than patients with RA in remission (DAS<2.6; p=0.008), but TTP did not differ significantly from that in patients with low disease activity (2.6<DAS28≤3.2). The TTP in patients with low disease activity did not differ significantly from that in patients in remission. (B) Patients using high prednisone dosages had a longer TTP than patients using low dosages (p=0.04), and a longer TTP than patients using no prednisone (p=0.002). The TTP between the low-dose group and the group with no prednisone did not differ significantly. DAS28, 28-joint Disease Activity Score.

Cox regression

Cox regression analysis with multiple variables showed that older age, nulliparity, higher DAS28, preconception use of NSAIDs, and preconception use of prednisone were associated with a longer TTP (table 3). Smoking, time since RA diagnosis, RF positivity, ACPA positivity, past MTX use, and preconception sulfasalazine use were not significantly associated with TTP.

When analysis was restricted to pregnancies resulting in a live birth, the same factors were identified as significantly associated with TTP (data not shown).

Because DAS28 and prednisone use may show interaction, we introduced an interaction term (DAS28 x daily prednisone dosage) into our model. This showed no significant effect (HR 1.00, 95% CI 0.99 to 1.02; p=0.41).

Table 2 – Differences between subfertile and fertile female patients with rheumatoid arthritis

Variable	Subfertility*		*p* value
	Yes (n=104)	No (n=141)	
Age, years	31.9±4.3	30.8±3.5	0.029 †
Nulliparity, n (%)	67 (64)	76 (54)	0.116 ‡
Smoking, n(%)	15 (14)	19 (13)	0.853 ‡
Duration of RA, years	3.7 (1.1-8.8)	3.4 (1.5-7.9)	0.589 ¶
RF positive, n (%)	83 (80)	97 (69)	0.058 ‡
ACPA positive, n (%)	76 (73)	85 (60)	0.042 ‡
Erosions present, n (%)	52 (50)	70 (50)	1.000 ‡
MTX in past, n (%)	67 (64)	94 (67)	0.786 ‡
Biologicals in past, n (%)	16 (15)	21 (15)	0.527 ‡
DAS28	4.01±1.16	3.47±1.10	<0.001 †
NSAIDs, n (%)	35 (34)	25 (18)	0.007 ‡
Prednisone, n (%)	46 (44)	35 (25)	0.002 ‡
Sulfasalazine, n (%)	35 (34)	44 (31)	0.782 ‡
Spontaneous abortion, n (%)	11 (11)	15 (11)	1.000 ‡

* Subfertility is defined as a time to pregnancy >12 months. Values are given as mean±SD, median (IQR) and no.(%).

† Student's t test.

‡ Fisher's exact test.

¶ Mann-Whitney U test.

ACPA, anti-citrullinated peptide antibodies; DAS28, 28-joint Disease Activity Score; MTX, methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; RA, rheumatoid arthritis; RF, rheumatoid factor.

Table 3 – Cox regression analysis for occurrence of pregnancy in female patients with rheumatoid arthritis

Variable	HR	95% CI	*p* Value
Age (per year)	0.96	0.92 to 1.00	0.038
Nulliparity	0.52	0.38 to 0.70	<0.001
Smoking	0.89	0.57 to 1.37	0.585
Disease duration (per year)	1.00	0.98 to 1.03	0.685
RF positivity	0.84	0.57 to 1.23	0.369
ACPA positivity	0.79	0.55 to 1.14	0.212
DAS28 (per point)	0.81	0.71 to 0.93	0.002
NSAIDs	0.66	0.46 to 0.94	0.022
Prednisone	0.61	0.45 to 0.83	0.002
Sulfasalazine	0.83	0.62 to 1.13	0.234
Past use of MTX	1.35	0.99 to 1.84	0.059

ACPA, anti-citrullinated peptide antibodies; DAS28, 28-joint Disease Activity Score; MTX, methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; RF,rheumatoid factor.

Prednisone

The effect of prednisone usage on subfertility was further assessed. Eighty-five patients used prednisone in dosages of 2.5–20 mg daily (median 7.5 mg). They were divided into two groups: a low-dose group (≤7.5 mg prednisone, n=44), and a high-dose group (>7.5 mg prednisone daily, n=41). In the high-dose group, 66% of women were subfertile compared to 43% in the low-dose group and 36% in the women who did not use prednisone. Kaplan-Meier curves showed a significant longer TTP in prednisone users versus non-users (p=0.005), and in high-dose users versus low-dose users (p=0.045) (figure 2B). In the Cox regression with the complete variable list, a dummy variable was introduced to distinguish between low-dose prednisone and high-dose prednisone. The HR for low-dose use was not significant (0.83, 95% CI 0.57 to 1.21; p=0.33), but use of high-dose prednisone significantly extended the TTP, with an HR of 0.50 (0.33 to 0.76; p=0.001). The significance of other variables in the analysis did not change.
A subgroup analysis on patients with DAS28 <3.2 still showed a significant association between prednisone use and a longer TTP (HR 0.21, 95% CI 0.10 to 0.45; p<0.001).

DAS28 after 1 year

A subgroup of women who did not conceive within 12 months after the first visit, were revisited after one year. In the 17 patients who were still actively trying to conceive, the DAS28 for this visit did not differ from the DAS28 1 year earlier (3.96±1.6 vs 3.91±1.8; p=0.85).

DISCUSSION

In this prospective cohort of female RA patients, we showed that a prolonged TTP in RA is related to older age, nulliparity, higher disease activity, and preconception use of NSAIDs and prednisone (>7.5 mg daily).
Forty-two percent of the patients had a TTP exceeding 12 months. This is much higher than the reported subfertility of 9-20% in the general population with a pregnancy wish in Western countries.[15,16] The median TTP of 6 months in pregnant RA women in our study is significantly longer than in the general Western European population, where 50% of women have a TTP of 3 months and approximately 70% conceive within 6 months after starting unprotected intercourse.[16]
The fertility rates in this study are similar to those shown by other studies in female RA populations of reproductive age. The proportion of 42% of subfertile women in

our study is consistent with a recent study that reported subfertility in 36% of women with RA diagnosed before family completion.[2] A Danish birth registry study found a TTP exceeding 12 months in 25% of pregnant RA women.[3] Since they did not include miscarriages or women who failed to conceive at all, these data are compatible with our study.

We identified various risk factors for a longer TTP in women with RA, including older age and nulliparity, which were already known to prolong the TTP. As in the general population, higher age negatively affects fertility.[17] The mean age in our patient group was 31.3±3.9 years, which is slightly older than the mean maternal age at child birth in the Netherlands (31.0 years in 2000–2010).[18] Therefore, we cannot explain the higher subfertility in this patient group by age alone.
Concerning nulliparity, it has been shown that a previous pregnancy increases the chance of a subsequent pregnancy, at least in subfertile couples.[19] In our study, 58% of patients were nulliparous compared to 47% in the general Dutch population.[4] Having a chronic disease may influence the time at which a woman starts having a family, or the choice to have fewer children, thereby explaining the higher number of nulliparous patients.[20] It seems unlikely that the higher number of nulliparous patients would reflect a selection bias, with relatively more subfertile women enrolling in our study, since the subfertility rate in this study is comparable with that reported in the literature.[2,3] Furthermore, it is not expected that this influenced the proper identification of RA-associated risk factors for a prolonged TTP in this study.

RA related factors that were associated with TTP in our cohort were DAS28, and preconception use of NSAIDs and prednisone >7.5 mg. To our knowledge, RA disease activity has not previously been related to reduced fertility, probably because of the retrospective design of previous studies. In inflammatory bowel disease, disease activity has been related to subfertility, but this is mainly attributed to tubal and ovarian dysfunction due to local inflammation or as a result of previous surgery.[21]
The impact of high RA disease activity on fertility could be mediated via inflammatory mediators, since many cytokines, chemokines and growth factors play an important role in the preimplantation blastocyst-endometrial interactions.[22] We have previously shown that high IL-6 serum levels are associated with lower birth weight in children born to women with RA.[23]

Since DAS28 did not increase during a 1-year follow-up in the preconception period, it is not likely that increasing disease activity over time explains an even longer TTP in these women.
The second factor is the use of NSAIDs. NSAIDs may interfere with ovulation, implantation and placentation through inhibition of prostaglandin synthesis.[10,24,25] Selective COX-2 inhibitors seem to inhibit ovulation more potently than traditional non-selective NSAIDs. However, this finding is only based upon case reports or small case series.[24]

Finally, the use of prednisone prolongs the TTP. Although prednisone has been considered not to have any effect on fertility when used for the treatment of chronic inflammatory diseases,[26,27] our results show that in daily dosages >7.5 mg it does indeed significantly lengthen the TTP. A possible explanation for this may be the transient suppression of the hypothalamic-pituitary-ovarian axis by glucocorticoids. Glucocorticoids in therapeutic dosages have been shown to decrease luteinising hormone pulse frequency from the pituitary gland.[28,29] Another possibility is a direct effect of prednisone on ovarian function or on the endometrium.[30-32]
Use of MTX in the past did not have a negative effect on the TTP. This is in contrast to animal studies, where MTX has been shown to cause a reduction in the number of primordial follicles (i.e. ovarian reserve) and a subsequent loss of ovarian function.[33] We have previously shown that short term MTX use in early RA does not affect ovarian reserve.[34] As the women in the current study had been using MTX for several years, our results suggest that long-term use of MTX also does not have a negative effect on ovarian function and fertility.

Even in the era of biologicals, our results are still relevant due to safety concerns regarding biologicals during pregnancy, and because not all women have access to them. Therefore, prednisone and NSAIDs are still important anti-rheumatic drugs during pregnancy and the preconception period.
It has been reported that women with inflammatory joint disease are more often nulliparous when diagnosed in early adulthood than when diagnosed in childhood or at a later age.[9] However, introducing age at diagnosis into our analysis has no significant effect on TTP (data not shown).
A reduced frequency of intercourse may also play a role in explaining the observed fertility problems. If a patient has chronic pain (eg, in the hip or knee joints), intercourse frequency is expected to be lower, thereby diminishing the chance of conception in a

given month. This was not assessed in our study, but after adjustment for DAS28, which reflects a patient's pain, other variables are still significant.
Body mass index (BMI) is also known to affect fertility. Overweight and underweight women both have a higher chance of ovulation disorders.[35] Overweight women with regular menses also have an increased risk of subfertility.[36] The association of RA with BMI is less clear.[37,38] BMI was not recorded for the women included in the PARA study, but the median BMI for women 18-42 years in a representative Dutch RA cohort was 24.2 (21.9-28.3).[34]

Based upon our results, it should be recommended that RA patients trying to conceive should strive for low disease activity, thereby avoiding NSAIDs and daily dosages of prednisone exceeding 7.5 mg. The treatment of some women in our cohort, reflecting common care for women with RA during the preconception period, does not seem to have been optimal as two-thirds of patients had a DAS28>3.2. While nearly a third of these women used no medication, over a third received monotherapy with sulfasalazine or prednisone. Combination therapy of sulfasalazine, prednisone and hydroxychloroquine in these patients may have resulted in lower disease activity.
Suppression of disease activity in RA women who wish to conceive is also important for the outcome of pregnancy. Higher DAS28 is associated with lower birth weight and rapid postnatal catch-up growth, which are both related to worse cardiovascular and metabolic profiles in adults.[4,39] Use of prednisone during pregnancy is associated with lower birth weight due to delivery at lower gestational age and with higher cortisol levels in the offspring.[4,40] Furthermore, women with high DAS28 more often undergo caesarean section.[4]

As the proportion subfertile women is much larger in the RA population than in the general population,[15] patients likely to have a longer TTP might be helped by early consultation with a gynaecologist on options for reproductive treatment. If the TTP can be limited in these patients, this may prevent extended suboptimal treatment and consequently functional disability and progression of joint damage.
The results of our study also have implications for patients with conditions other than RA. When patients wish to conceive and are using high daily dosages of prednisone, or NSAIDs on a regular basis, their treatment should be critically evaluated. Similarly in patients with other inflammatory auto-immune diseases, disease activity may impair fertility and should be suppressed whenever possible.

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Chapter 3

Subfertility in women with rheumatoid arthritis and the outcome of fertility assessments

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ABSTRACT

Objective: Subfertility is frequently encountered amongst female rheumatoid arthritis (RA) patients and has been associated with disease activity and anti-rheumatic drugs. However, little is known about the results of the fertility work-up in these women. Our aim was to study the outcome of fertility assessments in subfertile women with RA.

Methods: A cross-sectional study was performed in a nationwide cohort of female RA patients who were pregnant or were trying to conceive between 2002 and 2010 (PARA study). Patients who had given consent for future contact (n=260) received a questionnaire on reproductive history, fertility work-up and fertility treatments. Medical files were obtained from attending gynaecologists.

Results: A completed questionnaire was returned by 178 women (68%), of whom 96% had ended their efforts to conceive. Eighty-two subjects (46%) had at least one subfertile episode, and for 61 women a diagnosis for subfertility was available. Unexplained subfertility (48%) and anovulation (28%) were the most common gynaecological diagnoses, and both occurred more often in RA patients than reported in the general population. Women with unexplained subfertility more often used NSAIDs during the periconceptional period. Seventeen percent of all pregnancies were conceived after fertility treatments. Fertility treatments had equal or higher pregnancy rates in RA compared to other subfertile populations.

Conclusion: Unexplained subfertility is more often diagnosed in subfertile female RA patients than in the general population, and is related to periconceptional NSAIDs use. Despite the higher incidence of subfertility in women with RA, the outcome of fertility treatments in these women appears favourable.

INTRODUCTION

Fertility is compromised in women with rheumatoid arthritis (RA). They often have fewer children than they intended to have and they are more often nulliparous.[1,2] In 36-42% of female RA patients, diagnosed before family completion, the time to pregnancy (TTP) exceeds twelve months,[1,3] whereas in the general population, this is only the case in 10-17% of couples.[4-6] Since in most women with RA anti-rheumatic treatment has to be adjusted before they start trying to conceive, a longer TTP can result in a prolonged period with less adequately controlled disease and consequently an increased risk for permanent damage to the joints. Hence, understanding the underlying mechanisms of subfertility in RA, and treatment of these mechanisms whenever possible, would be an important step forward in the care for these patients.
Fertility in a couple might be compromised by many different factors, in the male as well as in the female partner. Male factors for subfertility include disorders in spermatogenesis or obstructions – causing oligospermia or azoospermia – and ejaculatory dysfunction. Female causes include anovulation, unilateral or bilateral tubal occlusion, and endometriosis. In 8-28% of subfertile couples no specific cause is found during fertility assessments. They are generally referred to as couples with unexplained subfertility.[5,7]
In most studies on fertility in RA, gynaecological data are missing. It has been reported that pregnant women with RA more often had a fertility treatment than pregnant controls.[8] However, little is known on the outcome of fertility assessments, and details on subfertility diagnoses have not been reported.
To study the outcome of fertility assessments in women with RA who suffer from subfertility, we performed a cross-sectional study in female RA patients from the Pregnancy-induced Amelioration of RA (PARA) cohort.

PATIENTS AND METHODS

Patients

Patients from the PARA study were invited to participate. The PARA study was a nationwide prospective observational cohort in the Netherlands (2002-2010) which included women with RA (1987 revised ACR criteria[9]) who were trying to conceive or were in their first trimester of pregnancy. PARA participants had to have a good understanding of the Dutch language and were allowed to participate more than

once. Both the PARA study and this follow-up study were approved by the Erasmus MC medical ethical review board. Of the 297 patients in the PARA cohort, 290 women gave permission to be contacted for future research. For 30 of them no current address was known.
For comparison of the occurrence of different subfertility diagnoses in women with RA to the general population, the incidence of these diagnoses in two reference populations with subfertility are reported.[5,7]

Data collection

Eligible patients received a printed questionnaire accompanied by a postage paid return envelope. Questionnaires included questions on their reproductive history, TTP in months and mode of conception for each pregnancy, visits to a gynaecologist, fertility assessments and fertility treatments.
When patients consented, data on fertility assessments were collected from the previously attended gynaecologists. The received files and letters were checked for menstrual cycle length, cycle irregularities and their classification (according to the World Health Organization (WHO) classification[10]), measurements of follicle stimulating hormone, oestrogens and progesterone, presence of ovulation, history of pelvic inflammatory disease, tests for tubal function (hysterosalpingography, laparoscopy), presence of endometriosis, abdominal adhesions and sperm analysis. The diagnosis was recorded. Furthermore, data on performed fertility treatments and their results were collected. When not all required data could be extracted from the received information, another letter was sent to the gynaecologist requesting explicitly for the missing items. In cases where the fertility work-up was not reported in full detail in the information received, we registered the diagnostic conclusion reported by the gynaecologist, assuming the work-up was performed according to the general consensus, as described in the national guideline by the Dutch Society for Obstetrics and Gynaecology (NVOG).[11] These guideline describes that in all couples consulting a gynaecologist for subfertility a fertility work-up includes at least: excluding anovulation by measuring a mid-luteal serum progesterone, assessing the male's fertility by performing a sperm analysis, and establishing Fallopian tube patency either by doing a Chlamydia antibody screening or by performing a laparoscopy or a hysterosalpingogram.

According to the national guideline (www.nvog.nl) unexplained subfertility was recorded when fertility assessments did not show any plausible explanation for the delay in the occurrence of a pregnancy.[12]
RA disease characteristics were drawn from the PARA study database,[13] including date of diagnosis, presence of rheumatoid factor (RF) or anti-citrullinated protein antibodies (ACPA), and disease activity scores.[13] Disease activity was scored using tender and swollen joint counts in 28 joints, and serum C-reactive protein levels (DAS28-CRP-3).[14]When available the preconceptional DAS28 of the designated PARA episode was used, otherwise the first trimester DAS28 of the same episode was used.[3]

Statistical analysis

To study potential selection bias, participants and non-participants were compared on obstetric history and disease characteristics as recorded during the last PARA episode that a subject had participated. Intergroup differences were calculated using the Student's t-test and Mann-Whitney U-test for continuous outcome and the Chi-squared test or Fisher's exact test for categorical outcome. Numbers of pregnancies, children and miscarriages were compared using a Mann-Whitney U-test.
The association of subfertility diagnoses with RA disease characteristics were studied using the Chi-squared test, Fisher's exact test or Student's t-test. Disease activity scores and periconceptional use of NSAIDs and prednisone from the first subfertile episode in the PARA study were used. A two-sided p-value <0.05 was considered significant. Software used was IBM SPSS statistics version 21 and STATA/SE 14.1 (StataCorp).

RESULTS

Participants

Of the 260 questionnaires sent, 178 (68%) were completed and returned (figure 1). Participants and non-participants were compared (table 1). Significantly more non-participants had not achieved a pregnancy during their most recent PARA study episode (37%) compared to participants (17%) (p=0.001). More often, non-participants were nulliparous at the end of their participation in the PARA study (24% versus 9%, p=0.002). There were more smokers among non-participants (22%) than in participants (6.7%) (p=0.001). Participants and non-participants did not differ significantly regarding age, education level, number of children they had already had, disease characteristics, or percentage of miscarriages or pregnancy complications.

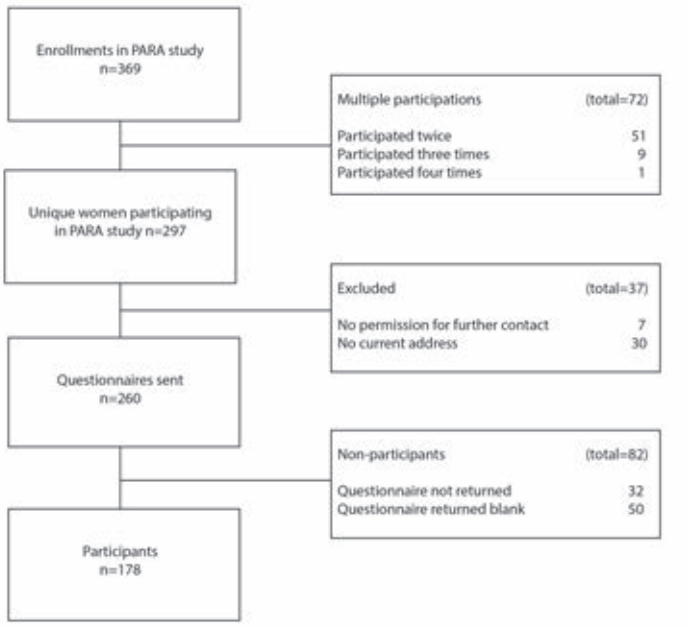

Figure 1. Flowchart of the study population and inclusion of study participants.

Reproductive history

Of the participants, 170 women (96%) had ended their efforts to establish a pregnancy. Reasons to stop trying to conceive were (multiple answers per subject possible): a complete family (72%), advanced age (20%), increase of RA complaints or the need for anti-rheumatic drugs incompatible with pregnancy (together 19%) or other health problems that complicated conception or taking care of a child (3%). In 9 women (5%) there were no more fertility treatments available or their gynaecologist advised against a new pregnancy. In 3% the mental burden of the wish to conceive was a reason to end their attempts at pregnancy, and in 1% relational problems played a role. In total 28% ended their efforts before they felt their family was complete. Fifty-nine women (33%) put their efforts to conceive on hold for one or more intervals, and in 32% of them this was due to RA complaints or the need for anti-rheumatic drugs incompatible with pregnancy.

The participants had a total of 412 pregnancies, with a median of 2 pregnancies (IQR 2-3, mean 2.3 ± 1.0) per woman. Of all reported pregnancies, 354 pregnancies (86%) were conceived after diagnosis of RA. Thirty-three percent of the women had one or more miscarriages (median 0, IQR 0-1, mean 0.42 ± 0.69). The total number of miscarriages was 75. There were 334 live born children in the study population

Table 1 - Characteristics of participants and non-participants

	Participants (n=178)	Non-participants (n=82)	*p*-value
Current age (years)	40.5 ± 4.2	40.6 ± 4.3	0.834 ŧ
missing	1 (0.6%)	-	
Education level			0.51§¥
Low	11 (6.2%)	5 (6.1%)	
Intermediate	74 (42%)	18 (22%)	
High	93 (52%)	28 (34%)	
missing	-	31 (38%)	
At start of last PARA episode			
Age during PARA (years)	32.4 ± 4.0	32.5 ± 4.0	0.905 ŧ
missing	2 (1.1%)	-	
Number of previous children	1 (0–1)	0 (0–1)	0.55β
mean	0.61 ± 0.63	0.59 ± 0.72	
missing	-	1 (1.2%)	
Smokers	12 (6.7%)	18 (22%)	<0.001ǂ
missing	2 (1.1%)	2 (2.4%)	
Duration of RA in years	15.0 ± 6.6	13.7 ± 5.9	0.135 ŧ
RF positivity **	129 (72%)	59 (72%)	0.826ǂ
missing	4 (2.2%)	1 (1.2%)	
ACPA positivity **	114 (64%)	55 (67%)	0.584ǂ
missing	1 (0.6%)	1 (1.2%)	
Periconceptional DAS28 **	3.7 ± 1.2	3.5 ± 1.3	0.394 ŧ
missing	17 (9.6%)	4 (4.8%)	
Obstetric outcome of last PARA participation			
Achieved pregnancy	147 (83%)	52 (63%)	0.001 ǂ
Life birth*	143 (97%)	50 (96%)	0.653 ¥
Miscarriages*	4 (2.7%)	1 (1.9%)	1.000 ¥
Maternal complications*	39 (27%)	12 (23%)	0.715 ¥
Neonatal complications*	24 (16%)	7 (13%)	0.824 ǂ
Nulliparous at end PARA study	16 (9.0%)	20 (24%)	0.002 ¥
missing	1 (0.6%)	2 (2.4%)	

Values are given as mean ± S.D., number(%), and median (interquartile range). * As a percentage of achieved pregnancies during last PARA participation. ** At the start of last PARA participation. P-values were calculated using ŧ unpaired T-test, ǂ Chi2 test, ¥ Fisher's Exact test (§ on non-missing data), and β Mann-Whitney U test. PARA=pregnancy-induced amelioration of rheumatoid arthritis; RA= rheumatoid arthritis; RF = rheumatoid factor; ACPA = anti-citrullinated protein antibodies; DAS28 = disease activity score with a 28 joint count.

(median 2 (IQR 1-2, mean 1.9±0.78) per woman), with a maximum of 4 children per woman. Nine women (5%) remained nulliparous, of whom six (3% of participants) had never been pregnant and three women (2%) had only had pregnancies resulting in a miscarriage.

Fertility assessments

Eighty-two women (46%, 95%CI 39–53%)) were considered subfertile because they met at least one of the following criteria: a TTP exceeding 12 months during at least one attempt to establish a pregnancy (n=66), the use of fertility treatments to get pregnant (n=41), or never having achieved a pregnancy (n=6). Sixty-six women (37%) had a primary subfertility, and 16 women (9%) suffered from secondary subfertility. The mean age during the first subfertile episode was 29.4 ± 3.9 years.

Subfertile women established significantly less pregnancies (2 (IQR 1-2, mean 2.1 ± 1.1) vs 2 (IQR 1-3, mean 2.5 ± 1.0), p=0.004) and had less children (2 (IQR 1-2, mean 1.6 ± 0.9) vs 2 (IQR 1-2, mean 2.1 ± 0.6), p<0.001) than fertile women with RA. The number of miscarriages per woman in both groups was not significantly different (p=0.33).

The total number of pregnancies in the subfertile women was 168, resulting in 132 life born children. Of these pregnancies, 52 (31%) were conceived spontaneously within 12 months, 48 spontaneously conceived pregnancies (29%) had a TTP >12 months, and 68 pregnancies (40% i.e. 17% of all pregnancies) were conceived with the help of fertility treatments (figure 2).

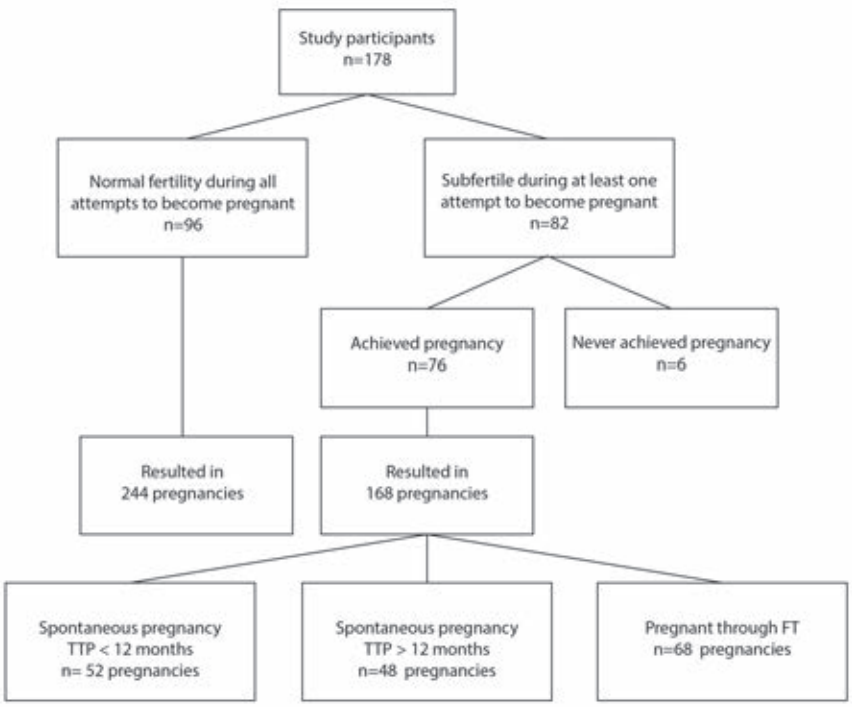

Figure 2 - Overview of study participants, pregnancies and subfertility. TTP = time to pregnancy; FT = fertility treatment

Fertility diagnoses

Eight subfertile women (10%) had never visited a gynaecologist. Of the other 74 subfertile women, 65 women (88%) gave permission to collect data from their gynaecologist. For 64 women data was obtained, of whom 61 women received a diagnosis for subfertility. The other 3 women became pregnant spontaneously before the fertility work-up was completed. The 61 women with a diagnosis were compared to the 21 subfertile women without available diagnosis, showing no significant differences regarding age, reproductive history, or disease characteristics. More women without available gynaecological files had ever used methotrexate (81% versus 54%, p=0.030).

In the 61 women for whom a diagnosis for subfertility was available, subfertility was most often caused by unexplained subfertility (48% of known diagnoses), anovulation (28%) and semen abnormalities (16%) (table 2). In 2 women (3%) anovulation was due to primary ovarian insufficiency (POI). In 5% of the couples there was both a female and a male cause for subfertility. For 8 of the 61 couples the gynaecologist did not report the result of a sperm analysis. Diagnoses in these women were: unexplained (n=2), vaginismus (n=1), anovulation (n=4) and endometriosis (n=1). For the 10 women for whom no gynaecology file was available, the self-reported diagnoses were: unexplained subfertility (n=4), tubal factor (n=2), anovulation (n=3, n=1 due to POI), and endometriosis (n=1) (table 2).

Of the women diagnosed by a gynaecologist (n=61), 56 (92%) had a subfertile episode in the PARA study, of whom 27 had unexplained subfertility and 14 had anovulation. When comparing women with unexplained subfertility to other subfertile RA patients, there was a significant difference in periconceptional NSAIDs use (48% vs 17%, p=0.013), which was also significant when self-reported diagnoses were included (NSAIDs use 45% vs 22%, p=0.046). None of the other associations, amongst which were disease activity and periconceptional prednisone use, were significant. Women with anovulation showed no statistically significant differences compared to ovulatory subfertile women.

Fertility treatments

The most frequently performed fertility treatments are summarized in table 3. Treatments were performed in 59 subfertile women (72%), which is 80% of the women who visited a gynaecologist for subfertility. In 41 women (50% of all subfertile women), fertility treatments resulted in at least one pregnancy, and in 28

of these women all conceptions were with the help of fertility treatments. Thirteen of the 20 women (65%) who started an in vitro fertilization (IVF) treatment had had intra-uterine inseminations (IUIs) before. Treatments not reported in the table are intravaginal insemination (1 subject), oocyte donation (1 subject), and sperm donation (2 subjects).

Table 2 – Fertility diagnoses in subfertile RA patients from the PARA study

Diagnosis	Diagnosis by gynaecologist (n=61)		Including self-reported diagnoses (n=71)		Reference population	
					Hull[5]	Thonneau[7]
	N	% of subjects	N	% of subjects	% of subjects	
Unexplained subfertility‡	29	48%	33	46%	31%	12%
Anovulation*/**	17	28%	20	28%	21%	32%
Male factor*	10	16%	10	14%	26%	58%
Endometriosis	4	6.6%	5	7.0%	6%	4%
Tubal occlusion*	2	3.3%	4	5.6%	14%	26%
Vaginismus	2	3.3%	2	2.8%	NA	NA

** In 2 couples there was both anovulation and a male factor. In 1 couple there was both tubal occlusion and a male factor.*

*** In 2 women anovulation was due to premature ovarian insufficiency (POI).*

‡ Unexplained subfertility includes a diagnosis of cervical hostility

Table 3 – Fertility treatment results in female rheumatoid arthritis patients from the PARA study

Treatment	Subjects	N of treatments			Resulted in pregnancy		Pregnancy rate	
	(n=55)*	sum	range	missing for n subjects	subjects (n=38)†	pregnancies (n=64)†	per cycle	per woman§
IUI**	36	178	(1-11)	3	12	19	11%	33%
OI	17	77	(1-12)	3	10	15	19%	59%
IVF	20	42	(1-4)	1	10	17	40%	50%
ICSI	8	25	(1-6)	-	8	15	60%	100%

IUI = intra-uterine insemination. OI=ovulation induction (with either clomiphene citrate or follicle stimulating hormone injections). IVF = in vitro fertilization. ICSI = intra-cytoplasmic sperm injection.

** 18 women had 2 types of treatments, 4 women underwent 3 different types of treatment.*

† 1 women achieved a first pregnancy after IUI, and a second pregnancy after IVF. 1 woman conceived twice after IUI combined with OI.

§ Percentage women with at least one pregnancy of total number of women who started treatment.

*** In 10 women IUI was combined with mild ovarian hyperstimulation (MOH). 9 pregnancies were after IUI with MOH.*

DISCUSSION

This study aimed to explore the outcome of fertility assessments in those RA patients with subfertility. We found that subfertility in women with RA was most often unexplained or caused by anovulation. Moreover, the majority of subfertile RA patients received fertility treatments, and a considerable number of all pregnancies were conceived after women had been treated for subfertility.

In comparison to the general population, female RA patients appear to be more often diagnosed with unexplained subfertility,[5,7,15,16] whereas the percentage of subfertile women with anovulation was equal or slightly increased compared to percentages found in the general population.[5,7,15,16] The incidence of primary ovarian insufficiency (POI) appears to be higher than in the general population.[17]

The high percentage of RA patients diagnosed by the gynaecologist with unexplained subfertility may imply that fertility in female RA patients is influenced by disease related factors. There was a significant association of periconceptional NSAIDs use with unexplained subfertility. This is in concordance with a previous study within the PARA cohort where we have shown that a longer TTP was associated with the periconceptional use of NSAIDs, also when corrected for disease activity scores.[3] In this previous study, a longer TTP was also associated with an increase in periconceptional disease activity and the periconceptional use of prednisone.[3] The current results did neither show significant differences in disease activity nor in periconceptional prednisone use between unexplained subfertile women and other subfertile RA women. However, since the current study was not designed to look into these associations, the numbers of patients in these comparisons were relatively small, and the timing of the study visit was not concurrent with the timing of the fertility work-up, these results do not exclude disease activity or prednisone use as a cause for subfertility in RA.

Looking further into the association of NSAIDs with subfertility, NSAIDs have not been reported to compromise embryo-implantation in IVF treatments,[18] but there have been reports on NSAIDs interrupting the ovulatory process, possibly leading to the so-called luteinized unruptured follicle (LUF) syndrome.[19-22] In LUF syndrome, ovulation is inhibited without changes in menstrual cycle length and cycle regularity and therefore patients with LUF would probably be classified as ovulatory during their fertility work-up. However, among gynaecologists the existence of LUF syndrome is controversial, and criteria for the existence of LUF syndrome include a laparoscopic

evaluation, with a strict timing, which is lacking in most studies on LUF syndrome.[23] Another explanation of the relation of NSAIDs use with unexplained subfertility may be the effect of pain on sexual intercourse. The use of NSAIDs may indicate that these women experience more pain and therefore they might have less exposure due to a decreased frequency of intercourse. Indeed, sexual intercourse in RA patients is more often limited by pain or fatigue,[24] but studies specifically addressing intercourse frequency in RA patients who try to conceive have not been performed. Furthermore, in daily practice RA patients who are trying to achieve a pregnancy are often very motivated to have regular peri-ovulatory intercourse. However, disease activity was higher in patients who used NSAIDs versus non-users, suggesting that NSAIDs were taken for pain control. All patients using NSAIDs reported that this was because of RA. No patient reported the use of NSAIDs for other conditions.

The percentage of subfertile subjects who received fertility treatments was almost fifty percent higher than in the general population.[4] Furthermore, the proportion of pregnancies that were conceived with the help of fertility treatments is conform earlier findings in the PARA study,[25] and is also in line with a Danish National Birth Cohort study reporting that more pregnant RA patients compared to pregnant controls had received a fertility treatment.[8] The pregnancy rates per treatment cycle and per woman who started treatment were comparable to other subfertile populations as far as intrauterine insemination (IUI) and ovulation induction (OI) were concerned.[26] On the contrary, the pregnancy rates of IVF and IVF/ICSI treatments were both higher in RA women.[27] Therefore, embryo implantation does not seem to be compromised in RA patients. An explanation for the higher pregnancy rate after IVF or ICSI might be explained by an early start of fertility treatment relative to their previous underexposure to preovulatory sexual intercourse. On the other hand, selection bias may have occurred when women who did not get pregnant after fertility treatments were mainly in the non-participants group. However, since data on the results of fertility treatments in these women were scarcely available, this remains unclear.

One in five women had stopped trying to conceive because of active disease or anti-rheumatic drugs. In the PARA study, more than one third of the women did not use any anti-rheumatic drugs during the periconceptional period, and less than 5% of the women used biologicals preconceptionally, whereas the disease activity was intermediate or high in the majority of patients.[3] Over the last decade, tumor necrosis factor inhibitors have been used increasingly in the periconceptional treatment

of women with RA, and appear to be safe.[28] Since active disease is associated with a longer TTP,[3] subfertile RA patients may benefit from the preconceptional use of these biologicals, and fewer women would need to end their efforts to build a family because of active disease.

With a higher pregnancy rate and less nulliparity than non-participants, the participants in this study seemed to be a more fertile selection of the PARA cohort. Therefore our current results may very well be an underestimation of the real incidence of the total subfertility and their impact on childbearing in the total female RA population. Since the incidence of subfertility in the PARA cohort was consistent with other recent studies in female RA patients of reproductive age,[3] it is not likely that selection on subfertility in the recruitment for the original PARA cohort introduced bias in the current study and affected our current results.

An explanation for the lower pregnancy rate and higher nulliparity among the non-participants may be that they were less desirous of achieving a pregnancy than the participants. However, this was not the case. No differences were found between participants and non-participants in the number of children already present before the final PARA participation. Moreover, within the non-participants who did not achieve a pregnancy during the last PARA episode more women underwent fertility treatments than the non-pregnant participants, although this difference was non-significant (data not shown). Therefore, the motivation to achieve a pregnancy appeared not to be diminished in the non-participants.

On the other hand, the cause of subfertility may have indirectly affected patients in their choice to participate in the current study. Fertility problems that are hard to treat, e.g. endometriosis, or a severe oligospermia or azoospermia, often have a poor treatment outcome, not leading to the desired pregnancy. Although the patients participating in the current study did not show a higher occurrence of these diagnoses than the general subfertile population, the non-participants were more often nulliparous. Therefore, we cannot rule out that the prevalence of these hard-to-treat cases might be more frequent in the non-participant group, and consequently also in the total RA population. Furthermore, there were more smokers among non-participants than among participants. This may point toward a less healthy lifestyle, which in turn is associated with reduced conception rates.[29] Details on smoking behaviour, like package years, are missing in the PARA study, and could not be analysed further. However, even if all non-participants of whom we know they had had fertility work-up or fertility treatments had another diagnosis than unexplained

subfertility, still the percentage of subfertile RA patients with unexplained subfertility would be higher than in the subfertile part of the general population.

In conclusion, our finding that unexplained subfertility is diagnosed more often in female RA patients than in the general population supports the idea that RA related factors are causally involved in subfertility in women with RA. Unexplained subfertility was associated with the periconceptional use of NSAIDs. Despite the higher incidence of subfertility in women with RA, the outcome of fertility treatments in these women appears favourable. In future research on fertility in RA, attention should also be given to intercourse frequency and sexual problems, since these are scarcely studied in RA patients trying to conceive. In daily practice, when an RA patient wishes to conceive, NSAIDs should be avoided, and early consultation with an expert rheumatologist and a fertility specialist should be considered to optimize the patient's chance of a complete family.

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Chapter 4

Miscarriages in female rheumatoid arthritis patients: associations with serologic findings, disease activity, and antirheumatic drug treatment

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ABSTRACT

Objective: To study the association between miscarriage in rheumatoid arthritis (RA) patients and serologic findings, disease activity, and antirheumatic drug treatment, and to study disease activity and reproductive outcomes after a miscarriage.

Methods: Within a nationwide prospective cohort study (Pregnancy-Induced Amelioration of RA study), patients with RA were followed up from preconception until 6 months after delivery or miscarriage. Univariate and logistic regression analyses were performed to assess variables of interest, with covariates included in the models if the *P* value for association with miscarriage was <0.20 and subsequently excluded if the *P* value was>0.10.

Results: Amongst 162 pregnancies, 28 miscarriages occurred (17.3%; 95% confidence interval 12.2-24.0%). Women who miscarried were older than women with an ongoing pregnancy. Women who miscarried tended to be more often positive for anti-citrullinated protein antibodies (ACPAs), to have higher disease activity scores, and to have more often received methotrexate (MTX) therapy in the past. Logistic regression showed a tendency toward a higher likelihood of miscarriage in association with increasing age (*P*=0.065), and presence of ACPAs (*P*=0.092).
After miscarriage, 33% of women had a flare of RA. Within 1 year, 68% of women became pregnant again, 14% stopped trying to conceive, and 11% were lost to followup. The Live birth rate of the subsequent pregnancy was 90%.

Conclusion: The miscarriage rate in the PARA cohort is comparable to that in the general population. Due to the low frequency of miscarriages in this study, the associations between miscarriage in RA and the presence of ACPAs, disease activity and MTX use did not reach statistical significance. Within 1 year after miscarriage, the majority of patients who continued trying to conceive achieved a pregnancy resulting in a live birth.

INTRODUCTION

Rheumatoid arthritis (RA) and female reproduction have been linked in the literature for decades. It has been shown that RA often attenuated during pregnancy, while disease flares occur during the postpartum period.[1] Furthermore, fertility is often impaired in female RA patients and is associated with disease activity and treatment with antirheumatic drugs.[2,3]

However, with regard to the frequency of miscarriages in patients with RA, results have varied greatly. Whereas some studies have not shown an increased miscarriage rate in RA patients,[4-6] others have demonstrated a significant increase in miscarriage rates both before and after disease onset.[3,7,8] Recently, a Norwegian birth registry study showed a significantly higher risk of miscarriage in women with RA compared to the general population.[8] The risk of recurrent miscarriage does not seem to be increased in RA, as no markedly increased numbers of miscarriages have been found in individual women.[5,7]

Miscarriages may be the result of disturbed inflammatory mechanisms. In humans, about one-half of embryo implantations do not result in a successful pregnancy. This may be attributed to not only genetic factors or metabolic abnormalities of the embryo but also to poor uterine receptivity.[9] A successful implantation may be facilitated by local injury of the endometrium, and more specifically, by an inflammatory reaction to such endometrial injury.[9] Therefore, inflammation, as well as antiinflammatory treatment, may play a role during implantation and early pregnancy in women with chronic inflammatory conditions such as RA.

Since miscarriages in RA have thus far been retrospectively assessed, no detailed information on patients' disease activity prior to conception has been reported. Moreover, reports on the preconception use of antirheumatic medications and rates of miscarriages may be biased. Therefore, the association between these factors and the occurrence of miscarriages in RA remains unclear. Miscarriages before the onset of RA are associated with more severe joint damage over time after diagnosis.[10] What happens to RA disease activity during the months directly after a miscarriage is not known.

Within a nationwide prospective cohort on pregnancy in RA, we studied the occurrence of miscarriage in women with RA and its association with serologic findings, disease activity, and periconceptional use of antirheumatic drugs. In addition, we assessed the course of RA after miscarriage and the occurrence of subsequent pregnancies.

PATIENTS AND METHODS

Patients

This study was performed within the Pregnancy-induced Amelioration of RA (PARA) study, a nationwide prospective cohort study in the Netherlands.[1] From 2002 to 2008, RA patients who wanted to become pregnant or were in their first trimester of pregnancy were invited by their attending rheumatologist to participate. Patients had to fulfill the American College of Rheumatology 1987 revised criteria.[11] According to current guidelines, patients were required to have stopped teratogenic medications, such as methotrexate (MTX), at least 3 months before trying to conceive to be eligible for the study. Informed consent was acquired from all patients. The study was approved by the Erasmus Medical Center medical ethics review board.

For the current study, all patients who were enrolled preconceptionally were included. If patients participated at least twice in the PARA study, only the first episode was included.

Data

During the study, patients were visited preconceptionally, during each trimester of pregnancy, and 3 times (6, 12, and 26 weeks) after delivery. When patients miscarried, they were also visited at 6, 12, and 26 weeks following their pregnancy loss.

Pregnancies were confirmed by the patients with a positive urine pregnancy test result. Miscarriage was defined as a loss of pregnancy before 16 completed weeks of gestation.

At each visit, patients were interviewed by a research team member to obtain information on obstetric history and medication use. The level of disease activity was measured using the Disease Activity Score in 28 joints (DAS28)[12] based on 3 variables: the swollen, tender joint count, and C-reactive protein (CRP) level.[1]

To determine the change in disease activity after miscarriage, we calculated the difference between the preconception DAS28 and DAS28 at 6, 12, and 26 weeks after miscarriage. We defined deterioration of RA as severe or moderate flares, defined as a reverse improvement response according to the European League Against Rheumatism response criteria for disease activity,[13] which is independent of the baseline DAS28.[1]

Finally, levels of CRP, presence of rheumatoid factor (RF) and presence of anti-citrullinated protein antibodies (ACPAs) were determined, as described previously.[2]

Statistical analysis

The primary outcome was the occurrence of a miscarriage before a gestational age of 16 weeks. The 9 variables of interest were maternal age at conception, presence of RF (at enrollment), presence of ACPAs (at enrollment), preconception DAS28, periconception use of nonsteroidal antiinflammatory drugs (NSAIDs) (daily or weekly during the preconception visit), use of sulfasalazine, use of prednisone, past MTX use, and total number of disease-modifying antirheumatic drugs (DMARDs) (current and past).

Differences between women who miscarried and women with an ongoing pregnancy were compared using Student's *t*-test or Fisher's exact test, as appropriate. Disease activity scores before conception and after miscarriage were compared using a paired *t*-test. Two-sided *P* values less than 0.05 were considered significant. Multivariate analysis was performed using logistic regression with purposeful selection of covariates, with a *P* value of <0.20 used as the significance level for inclusion, and subsequent exclusion of covariates based on a *P* value of >0.10.[14] The statistical package used was STATA/SE 13.1 (StataCorp).

RESULTS

Characteristics of the patients

There were 239 preconception visits within the PARA study, of which 58 did not result in a pregnancy within the followup period. Of the women who became pregnant, 13 participated twice and 2 women participated 3 times. Only their first study episode was used. Two patients were excluded: 1 patient had a molar pregnancy, and 1 patient had a pregnancy loss after intrauterine treatment during a twin pregnancy. There were 162 pregnancies for analysis.

The baseline characteristics of the patients are shown in Table 1. Eighty-two patients (50.6%) had already experienced at least one pregnancy, Of whom 22 (26.8%) had experienced one or more miscarriages in the past. During the analyzed study episode, 28 women (17.3%; 95% confidence interval 12.2%–24.0%) had a miscarriage: 14 miscarriages occurred in the second month of pregnancy (range 5.6–8.6 weeks), 9 miscarriages in the third month (9.3–11.9 weeks), and 5 miscarriages after 13 weeks of gestation (13.7–14.7 weeks). One woman had miscarried once before, and 1 woman had 3 previous miscarriages. There were no pregnancy losses between 16 and 20 weeks of gestation.

Table 1 - Baseline characteristics of the study patients (n = 162)

Variable	Value	Patients with missing variables, no. (%)
Age, mean ± SD years	32.3 ± 3.9	1 (0.6)
Never been pregnant, no. (%)	80 (49.4)	-
Previous miscarriages, no. (%)		
1	15 (9.3)	-
2	5 (3.1)	-
3	2 (1.2)	-
Smoking, no. (%)†	21 (13.0)	2 (1.2)
Duration of RA, median (IQR) years	4.9 (2.6 - 10.6)	2 (1.2)
RF positive, no. (%)	114 (70.4)	1 (0.6)
ACPA positive, no. (%)	103 (63.6)	1 (0.6)
Erosions, no. (%)‡	86 (53.1)	29 (17.9)
DAS28, mean ± SD	3.65 ± 1.14	1 (0.6)
Medication use, no. (%)		
MTX in past	114 (70.4)	-
NSAIDs periconception	47 (29.0)	-
Sulfasalazine periconception	54 (33.3)	-
Prednisone periconception	60 (37.0)	-
Cumulative DMARDs§		
None	17 (10.5)	-
1	42 (25.9)	-
2	54 (33.3)	-
3	34 (21.0)	-
≥4	15 (9.3)	-
Biologic drugs¶		
Past	23 (14.2)	-
Current	7 (4.3)	-

* RA = rheumatoid arthritis; IQR = interquartile range; RF = rheumatoid factor; ACPA = anti-citrullinated protein antibody; DAS28 = Disease Activity Score in 28 joints; NSAIDs = nonsteroidal antiinflammatory drugs.
† Smoking status (yes/no) during the preconception period, as reported by the patient.
‡ Presence of erosions, as reported by the patient.
§ Cumulative number of different disease-modifying antirheumatic drugs (DMARDs) taken prior to the preconception visit, including both conventional DMARDs (methotrexate (MTX), sulfasalazine, hydroxycholorquine, gold, prednisone, azathioprine, and leflunomide) and biologic agents.
¶ Use of biologic drugs (etanercept, adalimumab, anakinra, and infliximab) in the past, or current use of biologic drugs (etanercept, adalimumab, and anakinra) during the preconception period.

Univariate analysis

Univariate analyses with the 9 variables of interest showed that women who miscarried were significantly older (mean ± SD age 33.9 ± 3.9 years) than women with an ongoing pregnancy (age 32.0 ± 3.8 years; $P = 0.022$) (Table 2). A larger proportion of women who miscarried were ACPA positive compared to those who had not miscarried (82% versus 60%), although the difference was not significant ($P = 0.058$).

The DAS28 tended to be higher in women who miscarried (mean ± SD 3.92 ± 0.94 versus 3.59 ± 1.17 in women who had not miscarried; *P* = 0.166), and more women with a miscarriage had received MTX therapy in the past (82% versus 68%; *P* = 0.174). There were no significant differences in RF positivity, periconception use of NSAIDs, sulfasalazine use, prednisone use, and median cumulative number of DMARDs.

Table 2 - Univariate analysis of covariates in patients with rheumatoid arthritis who did and those who did not experience miscarriage*

	Miscarriage		
Variable	Yes (n = 28)	No (n = 134)	*P*
Age, mean ± SD years	33.9 ± 3.9	32.0 ± 3.8	0.022†
Missing, no.	0	1	–
RF positive, no. (%)	22 (79)	92 (69)	0.478‡
Missing, no.	-	1	–
ACPA positive, no. (%)	23 (82)	80 (60)	0.058‡
Missing, no.	-	1	–
DAS28, mean ± SD	3.92 ± 0.94	3.59 ± 1.17	0.166†
Missing, no.	-	1	–
Medication use			
MTX in past, no. (%)	23 (82)	91 (68)	0.174‡
NSAIDs periconception, no. (%)	6 (21)	41 (31)	0.371‡
Sulfasalazine periconception, no. (%)	10 (36)	44 (33)	0.827‡
Prednisone periconception, no. (%)	11 (39)	49 (37)	0.473‡
Cumulative no. of DMARDs, median (IQR)§	2 (1–3)	2 (1–3)	0.461¶

* RF = rheumatoid factor; ACPA = anti-citrullinated protein antibody; DAS28 = Disease Activity Score in 28 joints; MTX = methotrexate; NSAIDs = nonsteroidal antiinflammatory drugs; DMARDs = disease-modifying antirheumatic drugs; IQR = interquartile range.
† By Student's *t*-test.
‡ By Fisher's exact test.
§ Including biologic agents.
¶ By Mann-Whitney U test.

Multivariate analysis

We performed logistic regression analysis with the occurrence of miscarriage as the dependent variable and maternal age, ACPA positivity, DAS28 and previous MTX use as independent variables. Two patients who did not miscarry had missing variables and were excluded from the analysis. None of the variables showed a statistically significant change in the risk of miscarriage. Two variables (DAS28 and past MTX use) had a subsequent *P* value for association with miscarriage of >0.10, and were therefore omitted from the model. After omitting the DAS28 (*P* = 0.227) and subsequently excluding past MTX use (*P* = 0.113) as independent variables, none of the remaining variables (increasing age and presence of ACPAs) showed any statistically significant effect (*P* = 0.065 and *P* = 0.092, respectively) (Table 3).

Table 3 - Logistic regression analysis of associations with miscarriage in patients with rheumatoid arthritis*

Variable	OR	95% CI
Complete model		
Age per year	1.11	0.99–1.25
ACPA positive	2.49	0.86–7.20
DAS28 per point	1.27	0.86–1.88
MTX use in past	2.52	0.86–7.36
Reduced model		
Age per year	1.12	0.99–1.25
ACPA positive	2.47	0.86–7.08

* None of the odds ratios (ORs) were significant at $P < 0.05$.
95% CI = 95% confidence interval; ACPA = anti-citrullinated protein antibody; DAS28 = Disease Activity Score in 28 joints; MTX = methotrexate.

Followup after miscarriage

Twenty-one women were revisited after miscarriage. Six weeks after miscarriage, disease activity was measured in 19 women. The mean ± SD DAS28 was 4.3 ± 0.88, which was higher than the preconception DAS28 of 3.9 ± 1.0 in these women, but the difference was not significant ($P = 0.064$). Six of these women (32%) had experienced a moderate disease flare by 6 weeks after miscarriage. There were no severe flares. Nineteen women were visited again 12 weeks after miscarriage. At that time point, the mean ± SD DAS28 was 4.1 ± 1.3, and an additional woman had experienced a moderate flare, resulting in a total of 7 (33%) of 21 women who had experienced a flare of RA between the preconception period and 3 months after miscarriage. Two patients had to put their efforts to conceive on hold, because they had restarted treatment with MTX or etanercept. No new flares had occurred in the 10 women who were revisited 6 months after miscarriage.

After they miscarried, 4 women (14%) stopped trying to conceive and 3 women (11%) were lost to followup. All of the remaining women (21 (75%) of 28) conceived after the miscarriage. Nineteen women became pregnant again within 1 year after miscarriage. Two women achieved pregnancies after 20 and 47 months respectively. Two women (10%) experienced one more miscarriage, but thereafter each had a live birth in the subsequent pregnancy.

DISCUSSION

In this study, 17% of pregnancies in RA patients ended in a miscarriage. Women with RA who miscarried were older and tended to be more often ACPA positive, to have higher disease activity, and to more often have been treated with MTX in the past. After miscarriage, one-third of women experienced a disease flare. In total, 75% of women with a previous miscarriage conceived again and delivered a live-born baby. The miscarriage rate of 17% in the PARA cohort, which is comparable to that in the general population (11-22%),[15] is probably an underestimation of the true miscarriage rate in female RA patients. All pregnancies in our analysis were planned pregnancies, in contrast to those in prior retrospective studies, many of which often also included unplanned pregnancies, which were generally associated with a less healthy lifestyle. Moreover, unplanned pregnancies in RA may be associated with postconception use of teratogenic medications such as MTX, and therefore these patients have a higher risk of miscarriage.[16] In the PARA cohort, there is a known healthy cohort effect,[17] since the cohort contains fewer smokers and the education level is higher than that in the general population. Furthermore, a considerable number of women in this study had already had already had a previous pregnancy, most of which were ongoing. Therefore, the a priori chance of establishing a subsequent ongoing pregnancy was probably already higher in this selected group.

This is the first study that measured disease activity during the preconception period in women with a subsequent miscarriage. The association between an increasing DAS28 and the occurrence of miscarriage was not significant. However, since both ACPA positivity and previous MTX treatment, both of which are markers of more active disease, showed an association with the occurrence of miscarriage, it is likely that disease severity increases the risk of miscarriage in RA patients. This is also in concordance with current findings in the literature on embryo implantation and pregnancy loss, which describes physiological inflammatory reactions within the endometrium, a finding that seems important in attempting to achieve successful embryo implantation and decidualization.[9,18] Active RA may increase the risk of pregnancy loss through disturbance of decidualization. Moreover, repeated implantation failures due to immune imbalances may explain the association of active RA with subfertility.[2] Furthermore, the inflammatory reactions in the endometrium may also be altered by antirheumatic medications, although we did not find any statistically significant associations with antirheumatic treatments in this study.

MTX, a folic acid antagonist, can be a disruptor of fetal development, and therefore it can potentially increase the miscarriage rate. However, a recent study on pregnancy outcomes after MTX use showed that there was no increased risk of miscarriage after preconceptional MTX use.[16] To be eligible for the PARA study, patients who had been taking MTX had to stop the drug at least 3 months before trying to conceive. Therefore, it is unlikely that MTX itself has a causal relationship with the occurrence of miscarriage in this cohort.

The percentage of patients who had a flare of RA within 6 or 12 weeks after their miscarriage was comparable to the percentage of patients with a flare after delivery.[1] However, patients who miscarry do not benefit from the attenuation of RA that often occurs in the second and third trimesters of pregnancy.[1] Furthermore, many patients who delivered subsequently restarted or intensified antirheumatic drug treatment, whereas patients who had a miscarriage often could not be treated optimally, because they were again actively trying to conceive. Because of the increase in disease activity in a period of less- intensive antirheumatic treatment, flares after miscarriage may result in not only disability and long-term joint damage but also reduced fertility.[2]

Although the PARA study is thus far the world's largest prospective cohort on pregnancy in RA, the associations found did not reach statistical significance due to the relative low frequency of miscarriages in the study. This also limited the possibilities for multivariate analysis. Nevertheless, this is the only known cohort of female RA patients with clinical information, such as medication use and disease activity scores, available during the preconception period. Since the presence of ACPA, past use of MTX, and disease activity scores all tend to be associated with the occurrence of miscarriage, it is plausible that RA patients with more severe disease have a higher risk of miscarriage.

In conclusion, miscarriage rates in RA patients are comparable to those in the general population. The observed associations between miscarriages and the presence of ACPAs, disease activity, and MTX use were not significant. Within 1 year after miscarriage, the majority of patients who continued trying to conceive achieved a pregnancy resulting in a live birth.

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Chapter 5

Levels of serum anti-Müllerian hormone, a marker for ovarian reserve, in women with rheumatoid arthritis

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ABSTRACT

Objective: Fertility is reduced in women with rheumatoid arthritis (RA), even before diagnosis. This may be due to a diminished ovarian reserve. The current study examined serum levels of anti-Müllerian hormone (AMH), the most reliable endocrine marker for ovarian reserve, in early RA patients, and the influence of disease activity and methotrexate (MTX) use on AMH concentrations.

Methods: Serum AMH levels were measured in 72 women with recent-onset RA ages 18–42 years and compared to 509 healthy women. The association between AMH and rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), erosions, C-reactive protein (CRP) level, disease activity (Disease Activity Score in 28 joints (DAS28)), and use of MTX was assessed.

Results: At diagnosis, age-adjusted serum AMH levels did not differ significantly between patients and controls ($P = 0.254$). AMH levels were not related to the presence of RF ($P = 0.487$), anti-CCP ($P = 0.686$), or erosions ($P = 0.350$), and showed no significant correlation with CRP levels ($r = -0.207$, $P = 0.083$) or disease activity scores (DAS28, $r = 0.007$, $P = 0.955$). After 6 months of treatment, AMH levels in patients ($n = 53$) were lower than at the time of diagnosis ($P < 0.001$), but did not differ from controls ($P = 0.741$). There was no significant difference in AMH values after 6 months of treatment between patients who did ($n = 31$) or did not ($n = 22$) receive MTX ($P = 0.287$).

Conclusion: AMH levels in women with early RA are comparable to those of healthy controls, indicating that the reduced fertility in this patient group is not caused by diminished ovarian reserve. AMH levels are not affected by disease activity or by short-term MTX use.

INTRODUCTION

Rheumatoid arthritis (RA) can already be manifest in women during their reproductive years and might therefore reduce the ability to have children. Female RA patients experience more fertility problems compared to healthy controls, resulting in a longer mean time to pregnancy.[1-3] A reduced fertility seems to be present before diagnosis in female RA patients.[1] Since, on average, women with RA appear to reach menopause at an earlier age compared to controls,[1] the question is raised whether reduced fertility in women with RA is related to a compromised ovarian reserve.

In fertility clinics the ovarian reserve, which constitutes the number of primordial follicles, is estimated using serum anti-Müllerian hormone (AMH) levels. AMH is a member of the transforming growth factor (TGF)-β family and is produced in the ovary by granulosa cells of early developing follicles.[4] In both healthy and subfertile women, there is a strong correlation between serum AMH levels and the number of developing follicles in the ovaries, which declines with advancing age until the follicle pool is nearly depleted and the woman enters menopause. Due to this gradual decrease of quantity, which seems to go hand in hand with a decrease in quality of the oocytes harboured by the ovarian follicles, a woman will become infertile approximately ten years before she will enter menopause. The age at which a woman reaches menopause, as well as the preceding period of decreased fertility and infertility, varies greatly among women. At present, serum AMH is the most reliable predictor for the age at which a woman will enter menopause.[5]

Little is known about the effect of disease activity, parameters of inflammation or use of anti-rheumatic drugs on measurement of serum AMH levels.

Since RA patients who try to conceive use adjusted, and often less effective, medication, they frequently have increased disease activity. Thus far, no reports on the effect of RA disease activity on serum measurements of AMH are known.

Preferably, women with RA who want to conceive should be screened for increased risk of subfertility before alteration or cessation of medication, so they can be referred to a gynecologist in due time and the time to pregnancy will be as short as possible. Since many of these patients use methotrexate (MTX), nowadays the first choice drug in newly diagnosed RA, the effect of this potentially harmful drug on AMH levels should be studied.

The aim of this study therefore is to compare serum AMH concentrations between women with early RA and healthy controls, and to assess the influence of parameters of disease activity and the use of MTX on serum AMH levels in women with RA in childbearing age.

PATIENTS AND METHODS

Patients

Patient data were derived from the Rotterdam Early Arthritis Cohort (REACH), a prospective cohort study in the greater Rotterdam area which was started in July 2004 and is still ongoing.[6] Enrolled patients had joint complaints for less than 12 months, which were not explained by trauma or overexertion. They did not use any anti-rheumatic medication before enrolment. The Erasmus MC medical ethical review board approved the study protocol and a written informed consent was acquired before inclusion. Patients were examined at several visits, including the first visit (T0) and 6 months later (T6). Patient characteristics, general history, disease activity scores and drugs prescribed were registered. When available, serum samples for each visit were stored. X-rays of hands and feet were made at baseline and assessed for bony erosions.

For the current study, all women aged 18 to 42 years fulfilling the 2010 ACR/EULAR RA classification criteria were included.[7] Serum samples from T0 and T6 visits were used.

Controls

An existing control population of healthy females was used.[8] From this population, all women aged 18 to 42 years were selected (n=509). All controls had regular menstrual cycles ranging between 25 and 35 days or were proven fertile. Twenty-nine percent of controls had ever been pregnant, for 60% this is unknown. No additional fertility assessments were available. Controls did not use any hormones or oral contraceptives during the three months prior to blood sampling. In case of proven fertility, blood sampling was at least six months after delivery. Sampling was performed randomly during the menstrual cycle.[8]

Measurements

Disease activity was scored using the Disease Activity Score with a 28 joint count (DAS28). Serum samples were stored at -80° Celsius. Laboratory measurements included IgM-rheumatoid factor (RF) (enzyme-linked immunosorbent assay (ELISA)), anti-cyclic citrullinated peptide (anti-CCP), (Elia CCP on immunoCAP 250; Phadia Freiburg, Germany), C reactive protein (CRP) levels (mg/L) (local standards) and AMH levels (µg/L) (in-house double-antibody ELISA (commercially available as GenII Beckman Coulter, Beckman Coulter, Inc., Webster, Texas)). The range of the AMH standards used in the in-house AMH assay was 0.037-5 ng/mL.[8]

Statistics

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. Univariate analysis of covariance (ANCOVA) was performed to adjust for age differences between groups. Correlations between AMH and CRP and DAS28 were assessed by the partial correlation coefficient, correcting for age. Differences between T0 and T6 within subjects were studied using the paired samples t-test or the Wilcoxon signed ranks test. The proportional change in AMH levels between T0 and T6 was calculated as AMH(T6)/AMH(T0). Linear regression was performed to study the effect of different variables on proportional AMH change.

Reference curves for serum AMH levels in controls as function of age were calculated from a linear regression model using a natural cubic spline fitted on log-transformed AMH values of the control group.[8]

Values are represented as mean ± standard deviation or median (interquartile range). Two-sided p-values <0.05 were considered significant. Statistical package used is SPSS Statistics 18.0.0 (IBM Corp., Armonk, N.Y., USA).

RESULTS

Patients

Between July 2004 and February 2011 174 women aged 18 to 42 years were enrolled in the REACH. Of the enrolled women, 95 (55%) fulfilled the 2010 ACR/EULAR RA classification criteria. Three of them were diagnosed psoriatic arthritis, but still fulfilled the classification criteria and were included in the analysis. One patient had a history of unilateral ovariectomy because of severe endometriosis and was excluded from the study. The first (T0) and second (T6) visits were included in the analysis. Serum samples from T0 were available for 72 women whereas 53 of them had also serum stored at T6. The median time between the two visits was 182 (175-189) days.

The patients with available serum at T0 did not differ from patients without available serum (n=22) concerning age, BMI, symptom duration, CRP, or proportion of patients positive for RF, anti-CCP or erosions. At T0, included patients had a significantly lower mean DAS28 of 4.52±1.29 compared to 5.19±1.05 in patients with no serum available (p=0.032) (table 1). Information on obstetric history was available in 50% of patients. Twenty-five percent had ever been pregnant. No additional data on fertility problems were available.

Table 1. Patient characteristics at time of diagnosis*

	Patients for analysis	Patients without available serum**	
	(n=72)	(n=22)	*P*-Value
Age - years	35.1 (30.1–39.2)	34.4 (29.6–38.7)	0.681†
BMI - kg/m²	24.2 (21.9–28.3)	23.7 (22.1–32.7)	0.531†
Missing	2	2	
Duration of symptoms - days	115 (67–182)	124 (75–306)	0.500†
RF positive - no. (%)	33 (46)	8 (47)	1.000‡
Missing	-	5	
Anti-CCP positive - no. (%)	39 (54)	7 (41)	0.422‡
Missing	-	5	
CRP - mg/L	7.0 (3.3–16.0)	11.0 (4.0–20.0)	0.167†
DAS-28 - mean (± SD)	4.52 (±1.29)	5.18 (±1.07)	0.032§
Erosions positive - no. (%)	10 (14)	2 (9)	0.726‡

*Except where indicated otherwise, values are the median (interquartile range).
**Patients without available serum at T0 were excluded from further analyses.
BMI = body mass index, RF = rheumatoid factor, anti-CCP = anti-cyclic citrullinated peptide, CRP = C-reactive protein, DAS-28 = disease activity score with a 28 joint count.
P-values indicate differences between the two groups, as calculated by (†) Mann Whitney-U test, (‡) Fisher's exact test and (§) Student's t-test

AMH at time of diagnosis

At T0, the median age in patients was higher than in controls (35.1 (30.1-39.2) and 29.4 (23.8-34.3) years respectively, p<0.001). BMI did not differ significantly (24.2 (21.9-28.3) and 22.9 (21.2-25.7) kg/m², p=0.063). Median AMH values were 1.71 (0.81-4.39) µg/L in patients and 2.82 (1.64-4.38) µg/L in controls. When adjusted for age, AMH values did not differ significantly between patients and controls (p=0.254). Eight patients (11%) had AMH levels below the 10th percentile of controls (figure 1A).

There was no significant difference in AMH levels, adjusted for age, when comparing RF positive with RF negative women (p=0.487). No differences in AMH concentrations were found between anti-CCP positive and negative women (p=0.686). AMH levels were not significantly different when erosions were present (p=0.350). Finally, there was no significant correlation between AMH and either CRP (r=-0.207, p=0.083) or DAS28 (r=0.007, p=0.955).

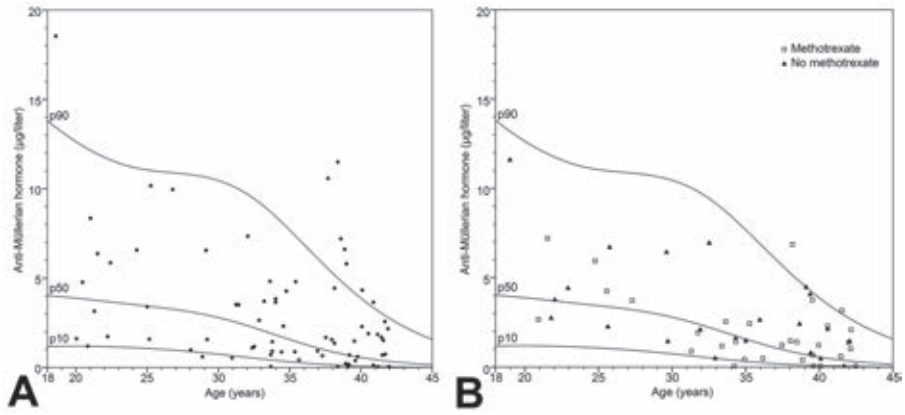

Figure 1. (A) Serum anti-Müllerian hormone (AMH) levels in women with recent onset rheumatoid arthritis at time of diagnosis (T0) are comparable to a healthy control population. (B) Serum AMH levels in women with recent onset rheumatoid arthritis six months after diagnosis (T6), differentiating between patients who did or did not receive methotrexate (MTX) treatment during these six months. The lines represent the 10th, 50th and 90th percentile lines of predicted AMH values in healthy controls.

AMH six months after diagnosis

Blood samples were available for both visits in 53 patients. The mean value of DAS28 decreased significantly during six months from 4.54±1.29 to 3.02±1.38 ($p<0.001$), as did CRP levels (7.0 (4.0-18.0) to 5.0 (2.0-9.4) mg/L, $p=0.008$).

AMH levels at T6 were significantly lower than at T0 (1.92 (0.84-3.75) and 2.57 (0.90-5.30) µg/L respectively, $p<0.001$). AMH levels at T6 were not different from those in controls ($p=0.741$). Five women (9%) had AMH levels below the 10th percentile of controls.

At T6, there was neither a significant correlation between CRP levels and AMH ($r=-0.170$, $p=0.247$), nor between DAS28 and AMH ($r=0.084$, $p=0.563$).

Thirty-one out of 53 women (58%) were prescribed MTX at the first visit in dosages of 7.5-25 mg/week (mean dosage 20 mg/week). These women had significantly higher CRP levels (8.0 (5.0-26.0) mg/L, $p=0.022$) and DAS28 values (4.91±1.38, $p=0.009$) at T0 than those who did not receive MTX (CRP 4.5 (2.0-11.0) mg/L and DAS28 4.04±0.96). The proportion of RF positive women was higher in the MTX group (68% versus 23%, $p=0.002$). At T0, age ($p=0.139$), BMI ($p=0.804$), symptom duration ($p=0.346$) and age-adjusted AMH levels ($p=0.229$) showed no significant difference between the two groups.

After six months of treatment, age-adjusted AMH levels did not differ significantly between MTX users and patients not using MTX ($p=0.287$). The proportional change

in AMH levels did not differ between the two groups (p=0.422) (figure 2), and was not dependent on age, DAS28, presence of RF or anti-CPP, and MTX use. AMH levels did not differ significantly either between patients using MTX and controls (p=0.394) or between patients without MTX and controls (p=0.657) (figure 1B).

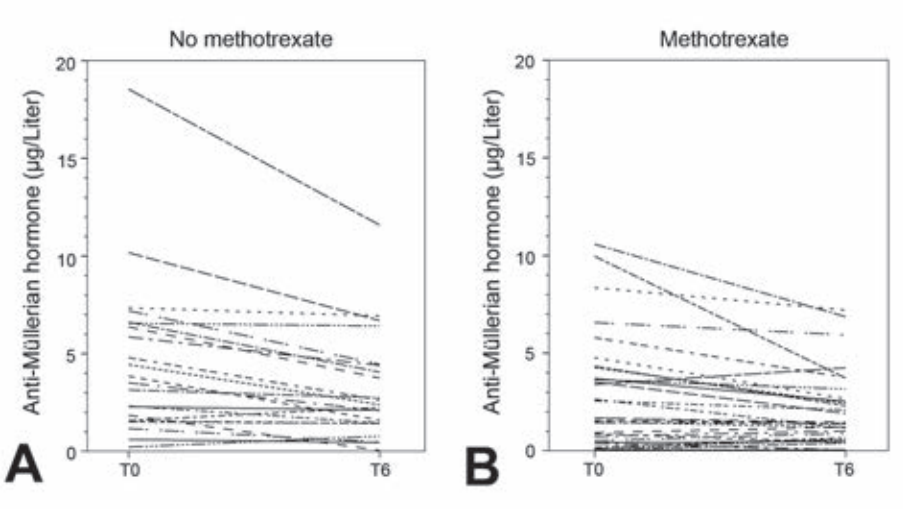

Figure 2. The individual change in serum anti-Müllerian hormone (AMH) levels from time of diagnosis (T0) to six months later (T6) in **(A)** rheumatoid arthritis (RA) patients not using methotrexate (MTX) and **(B)** RA patients who are using MTX during the six months following diagnosis. The proportional change in time of AMH levels between the two groups shows no significant difference (p=0.422).

DISCUSSION

To our knowledge, this is the first study reporting on AMH serum levels in RA patients. Our results show that serum AMH concentrations in women with early RA are comparable to healthy controls. Serum AMH levels in early RA are neither influenced by parameters of disease activity nor by use of MTX. This study hereby provides an answer to Clowse et al., who suggest AMH as a marker to detect subclinical damage to the ovary during and after cyclophosphamide therapy, but point out that the impact of rheumatologic disease and anti-rheumatic drugs on AMH expression is unknown.[9] Subfertility might already be present in RA patients at time of diagnosis.[1] However, according to current results, this appears not to be due to a reduced ovarian reserve. As earlier menopause has especially been reported in RF positive RA patients,[1] auto-

immunity could play an important role in the early exhaustion of the primordial follicle pool. In RA, auto-antibodies like RF and anti-CCP can be found years before the disease becomes manifest.[10] These auto-antibodies are thought to represent a subgroup of RA patients with most severe disease and the highest chance for extra-articular manifestations. Therefore, in case a decreased ovarian reserve might already be present early after diagnosis, it is most likely to be found in this subgroup of patients. The results of this study however, do not confirm the hypothesis of a decreased ovarian reserve at time of diagnosis in either anti-CCP or RF positive women.

Another possible explanation for early menopause is MTX, the first choice drug for newly diagnosed RA patients. Women with RA who want to become pregnant may have been using MTX for a considerable amount of time. However, there is a lack of studies on the effect of long term low-dose MTX treatment on ovarian reserve in humans. In rats, daily administration of low (0.05 mg/kg) or high dose (0.15 mg/kg) MTX for 20 days showed a dose dependent loss of vaginal cyclicity, and hormonal changes towards post-menopausal values. Ovarian preantral and antral follicle growth was reduced. This was already apparent in low dose MTX treatment and became even more distinct in rats that received higher doses.[11] However, in our study, there was no effect of MTX on AMH levels, suggesting that short term MTX does not affect ovarian reserve. Further research is needed to elucidate the effect of long-term low-dose MTX treatment on fertility in women with RA. Unfortunately, the number of premenopausal women in the REACH with a follow up of one or more years is too small to study the long term effects of MTX treatment on fertility in this group.

In current study, detailed information on obstetric and gynecologic history was available for a limited number of patients. Since it is reported that serum AMH levels are not altered by use of hormonal contraception or parity, this is highly unlikely that these missing data would affect the outcome of this study.[12,13] Timing of blood sampling in the menstrual cycle was not available, but it has been shown that serum AMH levels do not differ significantly throughout the menstrual cycle.[14]

In conclusion, the current study shows that AMH levels in RA patients are not influenced by disease activity and MTX use. Furthermore, the results do not confirm a reduced fertility based on a demise of the primordial follicle pool at time of diagnosis in women with RA, since the ovarian reserve, as measured by AMH levels, is comparable to the control group. Long term follow up of female RA patients should clarify the role of ovarian reserve and other clinical factors in subfertility in this patient group.

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Chapter 6

Anti-Müllerian hormone levels in female rheumatoid arthritis patients trying to conceive – the role of ovarian function in time to pregnancy in a nationwide cohort study

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To be submitted

ABSTRACT

Background: Subfertility, a time to pregnancy (TTP) longer than 12 months, is present in 40% of female rheumatoid arthritis (RA) patients who are actively trying to conceive. Since RA patients appear to reach menopause at a younger age, a lower ovarian reserve may explain the reduced fertility. Serum anti-Müllerian hormone (AMH) levels are the best proxy to measure ovarian reserve. Our objectives were to study AMH levels in female RA patients and determine the association of preconceptional serum AMH levels with TTP.

Methods: a post-hoc analysis was performed within patients of the Pregnancy-induced Amelioration of RA (PARA) cohort, who were assessed preconceptionally. Serum AMH levels were measured with the Ansh Labs pico AMH ELISA assay, and compared to an existing cohort of healthy controls.

Results: preconceptional serum was available in 209 women of the PARA cohort (aged 32.1±3.9 years), of whom 45% were subfertile in the current episode. The median AMH level was 2.5 ug/L (IQR 1.5–4.6). AMH levels were significantly lower compared to healthy controls ($p<0.001$), with 17% of patients having levels below the age-specific 10th percentile. Multivariable analysis showed a negative association of AMH with the presence of anti-citrullinated protein antibodies (ACPA) ($p=0.009$). AMH levels showed no significant association with TTP ($p=0.26$).

Conclusion: women with RA have lower AMH levels than healthy controls, and AMH levels were lower in ACPA positive patients. However, since preconceptional AMH levels were not associated with TTP, the reduced AMH levels do not explain the reduced fertility in RA patients.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory auto-immune disease that not only affects the joints, but also has extra-articular manifestations. RA can already be manifest at reproductive age and might therefore reduce the ability to have children. Approximately 40% of female RA patients suffer from subfertility – a time to pregnancy (TTP) longer than 12 months despite regular unprotected intercourse – compared to 10-15% of women from the general population.[1-3] Overall, RA patients have less children and are more often nulliparous than non-RA controls.[4]

A longer TTP in RA patients has been associated with older age, no previous pregnancies, higher disease activity, and the periconceptional use of NSAIDs and prednisone.[2] However, little is known about the ovarian function in RA patients and its effect on fertility. Since women with RA appear to reach menopause at a slightly younger age,[4,5] and since several autoimmune disorders have been related to primary ovarian insufficiency,[6,7] the ovarian follicle pool in female RA patients may be reduced at a relative early age. A recent small study has indeed reported results suggesting a reduced ovarian reserve in women with established RA.[8] Since in general a woman becomes less fertile approximately 10 years before she experiences menopause, a younger menopausal age is related to an earlier decrease in fertility,[9] reflected by a longer TTP or not achieving pregnancy at all.

The reduced fertility with increasing age is strongly correlated with the decline in number of follicles present in the ovary.[9] The size of the primordial follicle pool can be estimated by measuring serum levels of Anti-Müllerian hormone (AMH), which is specifically produced by the granulosa cells of the small growing ovarian follicles.[10,11] AMH levels are highest in early adulthood and decline with age, until they are undetectable around menopause.[12] Unlike other hormonal markers for ovarian function, such as follicle stimulating hormone (FSH), fluctuations in serum AMH levels throughout the menstrual cycle are small.[13] It should be noted that AMH expression in follicles may be downregulated, causing a discrepancy between AMH levels and the actual primordial follicle pool. For example, declined serum AMH levels during pregnancy have been reported.[14-17] Still, at present, a woman's serum AMH level is the most reliable predictor for the age at which she will enter menopause.[18] Regarding fertility, AMH levels have been reported to add to the prediction of live birth

in assisted reproductive technology cycles,[19-22] and low serum AMH levels have been described to be associated with a reduced chance of natural conception,[23] although conflicting results have been reported.[24]

To study the ovarian reserve in women with RA, we measured serum AMH levels in women with RA who had participated preconceptionally in a nationwide cohort study, and we studied the association of preconceptional AMH levels with subfertility in these women, as measured by TTP.

METHODS

Patients

A post-hoc analysis was performed within the Pregnancy-induced Amelioration of RA (PARA) study, a nationwide prospective cohort from 2002-2010 from the Netherlands. Patients were recruited in 2002-2008 by their attending rheumatologist, when they had a diagnosis of RA according to the American College of Rheumatology (ACR) 1987 classification criteria[25] and were trying to conceive or were in their first trimester of pregnancy. Patients had to have a good understanding of the Dutch language.[26] The PARA study was approved by the Medical Ethics Research Committee of the Erasmus MC, university medical center Rotterdam.

For the current study, only those patients were included in the analysis who were assessed during the preconceptional period. In patients who participated twice or more in the PARA study, only the first occurring preconceptional visit was selected.

Data collection

During the PARA study, patients were visited at their homes by a member of the research team. Assessments took place before conception (when possible), during each trimester of pregnancy, and 6, 12 and 26 weeks after delivery. At every visit, patients filled out questionnaires on obstetric history, medication use, health and daily functioning. Disease activity was measured, and a blood sample was drawn.[26]

All data was collected in the PARA database. Variables used for analysis were: date of start trying to conceive, age at start trying to conceive, age at drawing of serum sample, duration of disease, occurrence of any previous pregnancies, presence of rheumatoid factor (RF), presence of anti-citrullinated protein antibodies (ACPA), preconceptional disease activity, patient-reported erosions, preconceptional use of

prednisone or sulfasalazine, preconceptional use of non-steroidal anti-inflammatory drugs (NSAIDs), and use of methotrexate in the past.
Subfertility was defined as not achieving pregnancy after 12 months of trying to conceive.

Measurements

Disease activity was measured using a tender and swollen joint count for 28 joints, combined with serum C reactive protein (CRP) levels (DAS28-CRP).[27] Details on the measurement of RF and ACPA can be found in the original report on the PARA study.[26]
Serum samples were stored at -80°C. Serum AMH levels (ug/L or ng/ml) were measured using pico AMH assay developed by Ansh Labs (Houston,Texas, USA).[28] The Limit of Detection (LoD) was 0.0012 ug/L.

Controls

Serum AMH values were compared to those of an existing healthy control group consisting of 554 adult women aged 18-47 years, who had regular menstrual cycles, and of whom the majority was proven fertile.[12] Since serum AMH levels in the control group were reported as values measured with the GenII Beckman Coulter assay, a conversion factor was used to compare the AMH levels in the patient group to those in the controls:[29]
AMH (pico AMH assay in ng/mL) = $(1.45 * AMH_{Gen\ II}) + 0.32$

Statistics

Serum AMH levels were skewed to the right and were log-transformed for analysis. To make log-transformation possible, serum AMH levels below the LoD (0.0012 ug/L), were imputed by dividing the LoD by the square root of 2.[30] Numbers of missing values were reported. Missing values were imputed using multiple imputation with chained equations. All variables of interest (see Data collection) that had missing values were included in the imputation, as well as all other variables intended to be put into the Cox proportional hazard analysis. Because of the survival nature of the data, also the event variable _d (occurrence of pregnancy) and the Nelson Aalen estimator (estimation of baseline hazard $H_0(t)$) were included in the imputation procedure.[31] Being outcome variables, missing values of _d and the Nelson Aalen estimator were not imputed.
An analysis of covariance (ANCOVA) was performed to compare log-transformed AMH levels, corrected for age, between RA patients and controls. Associations of various

variables with AMH levels were tested separately using linear multiple regression on the log-transformed AMH values. A multivariable analysis was performed by entering all variables of interest into the multiple linear regression. Since AMH can be considered a proxy for ovarian age despite a woman's calendar age, the multivariable analysis was performed both with and without age as a covariate.

To study the TTP, a Kaplan-Meier curve was drawn. To avoid negative values of TTP for women who conceived within the first menstrual cycle, the fictitious date of the positive pregnancy test was calculated by adding 28 days to the first day of the final menstrual period. The Cox proportional hazard analysis was performed with the occurrence of pregnancy as event variable. Aside from log-transformed AMH levels and age, covariables were selected on the basis of our previous study on TTP in RA: never been pregnant before, disease duration, presence of RF and ACPA, DAS28, preconceptional use of NSAIDs, of prednisone and of sulfasalazine, and past methotrexate use. Smoking was not included, since the percentage of smokers in the PARA study was low and had no effect on TTP in the previous study.[2] Presence of erosions was added, as a measure for long term disease severity. In a second analysis, age was excluded, to study whether unadjusted AMH levels showed an association with TTP.

P-values below 0.05 were considered statistically significant. Statistical software used is STATA 14.1 (College Station, Texas, USA).

RESULTS

Patients

A preconceptional serum AMH was available in 209 patients within the PARA study (figure 1). Baseline characteristics and missing values are reported in table 1.

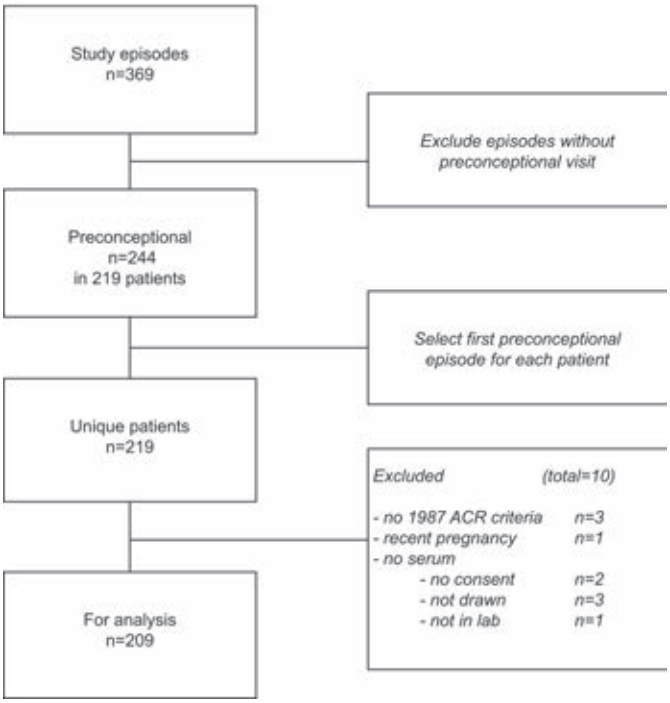

Figure 1 – flow diagram for available preconceptional patients within the PARA study

A pregnancy was achieved during follow-up after the preconceptional study visit in 159 women (76%). In 47 women (22 %) the episode did not result in a pregnancy, and 3 women (1.4%) were lost to follow-up without knowing whether a pregnancy was achieved. In the women who got pregnant, the median time to pregnancy since patients actively tried to conceive was 6.1 months (IQR 2.6 – 15.4 months). In women who did not achieve pregnancy, the median follow-up period was 17.3 months (IQR 14.4 – 25.8 months). A Kaplan-Meier curve for time to pregnancy is shown in figure 2.

Table 1 - Baseline characteristics

Variable	Preconceptional
	n=209
Age - years (mean ± sd)	
at start trying to conceive	31.7 ± 3.9
at study visit	32.1 ± 3.9
Previous pregnancies (n (%))	
0	110 (53%)
1	67 (32%)
2	25 (12%)
3 or more	7 (3.4%)
Previous miscarriages (n (%))	
0	181 (87%)
1	20 (9.6%)
2 or more	8 (3.8%)
Disease characteristics	
Duration of RA - years (median (IQR))	
at start trying to conceive	4.1 (1.7 - 9.0)
at study visit	4.5 (2.0 - 9.4)
missing	2 (1.0%)
RF positive (n (%))	153 (73%)
missing	2 (1.0%)
ACPA positive (n (%))	137 (66%)
Presence of erosions (patient) (n (%))	107 (51%)
missing	41 (20%)
DAS28-CRP at study visit (mean ± sd)	3.73 ± 1.17
missing	3 (1.4%)
Anti-rheumatic drugs use at time of study visit (n (%))*	
None	19 (9.1%)
Prednisone	89 (43%)
Sulfasalazine	76 (36%)
Hydroxychloroquine	16 (7.7%)
NSAIDs (incl COX2 inhibitors)	94 (45%)
Biologics	8 (3.8%)
Ever used MTX (n (%))	148 (71%)
missing	1 (0.5%)

RA = rheumatoid arthritis, RF = rheumatoid factor, ACPA = anti-citrullinated protein antibody, DAS28-CRP = disease activity score using a 28 joint count and C-reactive protein, NSAIDs= non-steroidal anti-inflammatory drugs, COX2 = cyclo-oxygenase 2;

** When using anti-rheumatic drugs, patients could be using more than 1 of the reported drugs.*

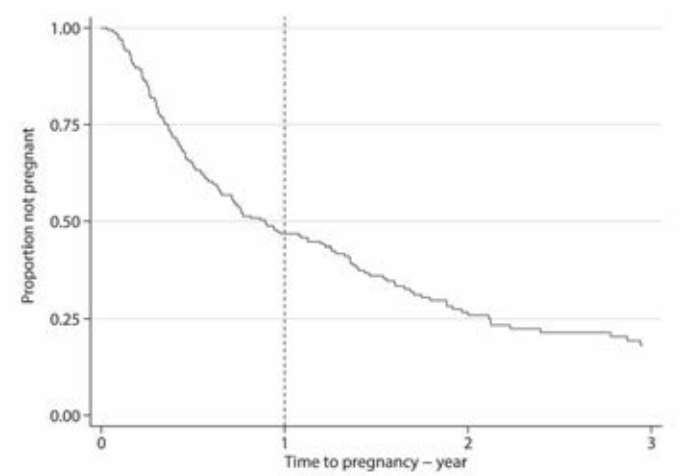

Figure 2 – Kaplan-Meier curve depicting the time to pregnancy in women with rheumatoid arthritis trying to conceive. T=0 is the moment indicated by the patients at which they first had actively tried to conceive.

Ninety-four women (45%) were subfertile based on a time to pregnancy >12 months or a follow-up time >12 months without achieving a pregnancy. For six women (2.9%) it was unknown whether they fulfilled the criteria for subfertility because it was unknown whether they achieved pregnancy during the follow-up (n=3), or because their follow-up time ended before they had been trying to conceive for 12 months (n=3).

Serum AMH levels and RA disease characteristics

The median preconceptional serum AMH level was 2.5 ug/L (IQR 1.5 – 4.6), with 1 woman having an AMH level below the LOD. The individual preconceptional serum AMH levels were plotted in the nomogram created for healthy controls[12] (figure 3). In 36 women (17.2%) the AMH levels were below the 10th percentile for their age, in 94 patients (45.0%) AMH levels were between the 10th and 50th percentile, in 73 patients (34.9%) between the 50th and 90th percentile, and in 6 (2.9%) of patients AMH levels were above the 90th percentile. ANCOVA with log-transformed AMH levels, showed significant lower AMH levels, corrected for age, in patients compared to controls (p=<0.001).

Mono-variable analyses showed a significant negative association of log-transformed AMH levels with increasing age (β= -0.070 (95%CI -0.11 – -0.031), p=0.001) and the presence of ACPA (β= -0.38 (95%CI -0.71 – -0.056), p=0.022) (table 2). Serum AMH levels were not associated with never having been pregnant before, disease duration, presence of RF, DAS28-CRP, presence of erosions, use of NSAIDs, use of prednisone,

sulfasalazine use, or past use of methotrexate. A multivariable linear regression of log-transformed AMH levels including all of the above covariates, showed a significant negative association with the presence of ACPA, both corrected for age (β= -0.47 (95%CI -0.89 – -0.051), p=0.028), as well as uncorrected for age (β= -0.57 (95%CI -0.99 – -0.14), p=0.009) (table 2).

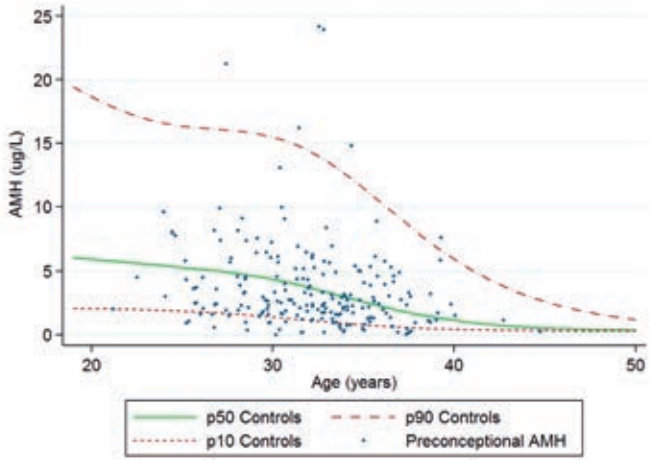

Figure 3 – Preconception serum AMH levels in 209 women with rheumatoid arthritis trying to conceive, plotted against the 10th, 50th and 90th percentile of serum AMH values in healthy controls.[12]

AMH and time to pregnancy (TTP)

For the Cox proportional hazard analysis, the preconceptional study visit at which the first serum was drawn was considered as the start of follow-up. Of the 209 women, 205 women were included in the analysis. Four women were excluded because it was unknown whether pregnancy had occurred (n=3) or what the exact follow-up time was (n=1). Log-transformed AMH levels, corrected for age, were not significantly associated with TTP (HR 1.09 (95%CI: 0.94–1.27), p=0.26). A longer TTP was associated with older age (HR 0.96 per year (95%CI: 0.91 – 1.00), p=0.052), never having been pregnant before (HR 0.43 (95%CI: 0.30 – 0.62), p<0.001), increasing disease activity (HR 0.85 per point of DAS28 (95%CI 0.73 – 0.98), p=0.026), and preconceptional NSAIDs use (HR 0.5(95%CI: 0.40 – 0.81), p=0.002) (table 3). An interaction term between age and AMH was not significant (p=0.20), and was not included in the model. When leaving age out of the model, the uncorrected AMH levels also showed no significant association with TTP (HR 1.14 (95%CI: 0.98 – 1.33), p=0.093) (data not shown).

Table 2 - The association of anti-Müllerian hormone (AMH) levels (log-transformed) with disease related factors in women with rheumatoid arthritis (n=209)

	Association with log(AMH)								
Variable	Beta	95% confidence interval			Beta	95% confidence interval			
Monovariable associations	*Uncorrected for age*				*Corrected for age*				
Age - per year	-0.070	-0.11	–	-0.031	**	-			
Never been pregnant before	0.16	-0.15	–	0.47		0.33	-0.28	–	0.35
Disease duration - per year	-0.022	-0.048	–	0.004		-0.018	-0.044	–	0.0074
RF positivity	-0.040	-0.40	–	0.32		0.033	-0.32	–	0.38
ACPA positivity	-0.38	-0.71	–	-0.056	*	-0.29	-0.61	–	0.036
DAS28-CRP - per point	-0.012	-0.15	–	0.12		-0.0020	-0.13	–	0.13
Presence of erosions	-0.24	-0.61	–	0.14		-0.25	-0.61	–	0.11
NSAIDs preconceptional	0.040	-0.28	–	0.36		0.021	-0.29	–	0.33
Prednisone preconceptional	0.031	-0.29	–	0.35		0.014	-0.30	–	0.32
Sulfasalazine	0.14	-0.18	–	0.47		0.15	-0.17	–	0.47
Past use of methotrexate	0.053	-0.29	–	0.40		0.036	-0.30	–	0.37
Multivariable analysis	*Uncorrected for age*					*Corrected for age*			
Age	-					-0.061	-0.10	–	-0.020	**
Never been pregnant before	0.13	-0.19	–	0.44		0.22	-0.30	–	0.34	
Disease duration - per year	-0.022	-0.053	–	0.0076		-0.18	-0.047	–	0.012	
RF positivity	0.30	-0.15	–	0.76		0.30	-0.14	–	0.75	
ACPA positivity	-0.57	-0.99	–	-0.14	**	-0.47	-0.89	–	-0.051	*
DAS28-CRP - per point	0.001	-0.14	–	0.14		0.0080	-0.13	–	0.15	
Presence of erosions	-0.085	-0.53	–	0.36		-0.14	-0.57	–	0.29	
NSAIDs preconceptional	0.010	-0.33	–	0.35		-0.001	-0.33	–	0.33	
Prednisone preconceptional	-0.0004	-0.34	–	0.34		-0.010	-0.34	–	0.32	
Sulfasalazine	0.17	-0.16	–	0.50		0.18	-0.15	–	0.50	
Past use of methotrexate	0.11	-0.25	–	0.47		0.095	-0.26	–	0.45	

AMH = anti-Müllerian hormone (in ug/L), RF = rheumatoid factor, ACPA = anti-citrullinated protein antibodies, DAS28-CRP = disease activity score with a 28 joint count and C-reactive protein, NSAIDs = non-steroidal anti-inflammatory drugs.

** p<0.05 ; ** p<0.01*

DISCUSSION

In female RA patients trying to conceive, we found that preconceptional serum AMH levels, corrected for age, were lower than those in healthy controls. AMH levels were significantly negatively associated with ACPA positive disease. Serum AMH levels did not show a significant association with the TTP in RA patients, not even when used as a substitute for age.

Table 3 - Cox regression for time to pregnancy counting from the moment of AMH measurement in women with rheumatoid arthritis who are trying to conceive (n=205)

Variable	Hazard Ratio	95% confidence interval	*p*-value
log(AMH)	1.09	0.94 – 1.27	0.26
Age - per year	0.96	0.91 – 1.00	0.052
Never been pregnant before	0.43	0.30 – 0.62	<0.001
Disease duration - per year	0.99	0.96 – 1.02	0.50
RF positivity	0.73	0.47 – 1.15	0.17
ACPA positivity	1.12	0.71 – 1.76	0.62
DAS28-CRP - per point	0.85	0.73 – 0.98	0.026
Presence of erosions	1.57	0.87 – 2.85	0.13
NSAIDs preconceptional	0.57	0.40 – 0.81	0.002
Prednisone preconceptional	0.77	0.54 – 1.10	0.16
Sulfasalazine	0.89	0.64 – 1.26	0.52
Past use of MTX	1.12	0.77 – 1.62	0.55

AMH = anti-Müllerian hormone (in ug/L), RF = rheumatoid factor, ACPA = anti-citrullinated protein antibodies, DAS28-CRP = disease activity score with a 28 joint count and C-reactive protein, NSAIDs = non-steroidal anti-inflammatory drugs, MTX = methotrexate

The lower AMH levels in RA patients confirm recent findings in a small study on AMH levels in RA, where 33 RA patients had lower AMH levels than age-matched controls.[8] On the other hand, at the time of RA diagnosis, AMH levels have been found not to be significantly different from controls.[32] Apparently, during the course of the disease, RA has a negative effect on the AMH levels. This is in line with studies on other chronic conditions reporting reduced AMH levels in women with cystic fybrosis,[33] girls with newly diagnosed cancer,[34] and women with Crohn's disease[35] or systemic lupus erythematosus.[36] Also in women with type 2 diabetes mellitus a reduced ovarian reserve has been reported, as reflected by elevated FSH levels and reduced antral follicle counts compared to controls.[37] Overall, these findings support the hypothesis that the ovarian function is affected by a woman's overall health status. Apparently, a woman's capacity to reproduce is compromised in times when the soma is less healthy. In women with RA, this is also reflected in the younger age at which they appear to reach menopause, as has been reported in a previous case-control studies.[4,5] Lower AMH levels, and the subsequently higher chance for early menopause may not only have implications for a woman's fertility, but also for her overall long term health, since women who reach menopause at a younger age have an ongoing unfavorable health state, with increased lifetime risk for conditions such as osteoporosis, and cardiovascular disease.[6]

In agreement with the other study on AMH in established RA,[8] we did not find a significant association of AMH levels with disease duration. However, we did observe a significant association of lower AMH levels with the presence of ACPA, which has been related to a more severe disease responding poorer to anti-rheumatic therapy.[38] Possibly women with more severe RA have high levels of circulating cytokines or immune cells affecting the ovaries during active disease, or the long-term use of anti-rheumatic drugs, such as methotrexate, affects the ovaries.[39] However, no significant effect of past use of methotrexate on TTP in RA has been reported.[2] Therefore, it will be interesting to analyze the effect of circulating cytokines and cumulative anti-rheumatic drug use on AMH levels in women with RA in future studies.

The comparison of AMH levels with controls has to be interpreted with caution. As explained in the methods section, the AMH levels in controls were previously measured using an assay by another manufacturer, and a conversion factor was applied.[29] The use of conversion formulae for comparison of AMH levels from different assays has been debated. Especially with higher AMH levels, the precision of using regression equations decreases.[29] However, the number of patients in our study with AMH levels around or above the 90th percentile of controls were low. Furthermore, the focus of our current report is on the lower AMH levels, and therefore less precision loss is to be expected. Indeed, using other conversion formulae that have been reported[29] still showed significantly lower AMH levels in RA patients compared to controls. Another point of consideration is the lower limit of detection for the applied pico AMH assay. Replacement of very low AMH levels in our cohort with the conversed limit of detection of the older AMH assay used in the control group still showed significant lower AMH levels in RA patients compared to controls ($p<0.001$, data not shown).

Although AMH levels in RA patients were lower than in healthy controls, serum AMH levels did not show a significant association with TTP. The reduced AMH levels may be explained by a diminished number of ovarian follicles, but may also be the result of a reduced expression of AMH by the individual follicles. Either way, the reduced AMH levels in women with RA do not appear to have a significant effect on their conception chances. In a previous report that has shown low AMH levels to be a predictor of reduced fertility, selected patients were older, had been trying to conceive for a shorter period of time, pregnancy rates were higher, and AMH levels were dichotomized for analysis.[23] Another report on healthy women of a younger age showed no reduced fertility when AMH levels were low.[24] On the contrary, it did report an association of

high AMH levels with a longer TTP, which could be explained by patients with high AMH levels more often suffering from anovulation or other characteristics of the polycystic ovary syndrome, which is known to cause subfertility.[24] In our study, AMH levels as a continuous variable showed a non-significant shorter TTP with increasing AMH levels. Splitting the AMH levels in quintiles comparing the lowest and highest AMH groups to the middle group, did not confirm the findings of these previous studies (data not shown).
Although AMH levels have not clearly been shown to predict a woman's TTP, many studies have shown that AMH levels are a reliable indicator for ovarian response after stimulation for artificial reproductive techniques, such as in vitro fertilization (IVF).[40-42]. No studies on ovarian stimulation results in RA patients have been available thus far, but given the current results, patients with established RA undergoing IVF may have a lower oocyte yield than would be expected by age alone.

As we have shown previously, disease activity and the preconceptional use of NSAIDs, did have a significant effect on TTP, as did not having had a previous pregnancy.[2] However, in the previous study also periconceptional use of prednisone had a significant effect on TTP, which we could not confirm in the current analysis. The estimated hazard ratio for the occurrence of pregnancy in prednisone users versus non-users in the current study appeared higher than in the previous study, also when repeating the analysis with the same covariates as in the previous study (data not shown). A possible explanation for this difference lies in the selection of patients. Where the former study included also first trimester visits, the current study only included preconceptional visits. This selection was chosen, because AMH levels are known to be decreased during pregnancy.[14-17] Comparing the baseline characteristics for our two studies, significantly more patients in the current study used prednisone or NSAIDs during the periconceptional period, and significantly less women used no anti-rheumatic drugs. Since TTP and the percentage of women without a previous pregnancy did not differ between the two studies, our current cohort does not seem to represent a less fertile selection than the previous study. Therefore, it is difficult to give a clear explanation for the difference in effect of prednisone on TTP. The advantage of the current exclusively preconceptional cohort is that such a study probably suffers less from recall bias of TTP and preconceptional use of anti-rheumatic drugs. Furthermore, possible effects of pregnancy on disease activity and serum AMH levels are excluded in this cohort.

Concerning disease characteristics, our study cohort appears to be representative for patients with established RA. However, we have to consider the changes in management of RA since the end of the PARA study follow-up in 2010. Nowadays, anti-rheumatic treatment is based on treat-to-target guidelines. Moreover, so-called biologicals (such as tumor necrosis factor alpha blockers) are prescribed more often and also earlier in the disease course. Since several biologicals appear to be safe during pregnancy,[43] they are also prescribed more often in women trying to conceive. Therefore, the current preconceptional patients will differ from the patients in the PARA cohort regarding disease activity and the use of anti-rheumatic drugs. New observational cohorts will have to address whether these changes in treatment have a positive effect on ovarian reserve, fertility and offspring in RA patients.

In conclusion, serum AMH levels are decreased in women with RA, and lower AMH levels show an association with ACPA positive disease, which is often related to a more severe disease state. However, preconceptional AMH levels are not associated with TTP in RA, and therefore cannot explain the reduced fertility in these patients. Since decreased AMH levels may result in an early occurrence of menopause, further study in RA patients should clarify the long-term effect of RA and anti-rheumatic drugs on the age at which menopause occurs.

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Chapter 7

Decline of ovarian function in patients with rheumatoid arthritis: serum anti-Müllerian hormone levels in a longitudinal cohort

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To be submitted

ABSTRACT

Background: In several chronic conditions an early decline of ovarian function is found. Whether the same holds true for chronic inflammation, such as rheumatoid arthritis (RA) is unknown. RA is known to compromise female fertility, and often affects women in their fertile age. Serum anti-Müllerian hormone (AMH) levels are a proxy for the total number of primordial follicles, and a reliable predictor of the age at menopause. Our objective was to study the longitudinal intra-individual decline of serum AMH levels in female RA patients.

Methods: Female RA patients from a nationwide prospective cohort (PARA study, 2002-2008), were re-assessed in 2015-2016. Serum AMH levels were measured using the picoAMH assay and compared to healthy controls. A linear mixed model (LMM) was built to assess the effect of RA-related clinical factors on the decline of serum AMH levels.

Results: One hundred and twenty-eight women were re-assessed at an age of 42.6 ± 4.4 years, with a median disease duration of 15.8 (IQR 12.7 – 21.5) years. The time between the first and last AMH assessments was 10.7 ± 1.8 (range 6.4 – 13.7) years. Participants represented a more fertile selection of the original PARA cohort. At follow-up, 39% of patients had AMH levels below the 10th percentile of controls (95%CI 31 – 48%), compared to baseline levels (16%; 95%CI 9.3 – 22%). The LMM showed a significant decline of AMH levels with increasing age, but no significant effect of RA-related factors on AMH.

Conclusion: AMH levels in RA patients showed a more pronounced decline over time than expected, supporting the idea that also in chronic inflammatory conditions, reproductive function is compromised, resulting in a faster decline of ovarian function over time and probably an earlier age at menopause.

INTRODUCTION

The ovarian function depends on both the quantity as well as the quality of the primordial follicles in the ovaries. At birth, there are approximately 1,000,000 primordial follicles present in the ovaries. This number gradually decreases over time, resulting in about 300,000 remaining follicles at menarche.[1,2] During her twenties and thirties, a woman's follicle pool further decreases, with a slight acceleration in her late thirties and early forties. Finally, the near depletion of the ovarian follicle pool is marked by the cessation of menstruation.[3] The last menstrual period in a woman's life span, i.e. the menopause, occurs at a mean age of 51 years. Infertility generally sets in approximately 10 years before a woman experiences menopause.[3] This results not only from the decline in number of developing ovarian follicles over time, but also from a decrease in quality of the oocytes maturing within these follicles due to accumulated damage from birth onwards.[4]

The age at which a woman reaches menopause, as well as the preceding period of decreased fertility and infertility, varies greatly between women.[3] At present, the serum level of anti-Müllerian hormone (AMH) is the most reliable predictor for the age at which a woman will enter menopause.[5] Although, prediction of the actual age at menopause based on a single AMH measurement is still not very precise.[5] AMH is a member of the transforming growth factor β family and is produced in the ovary by granulosa cells of early developing follicles.[6] In both healthy and subfertile women, there is a strong correlation between serum AMH levels and the number of developing follicles in the ovaries. It has been shown that serum AMH levels become undetectable approximately 5 years before a woman reaches menopause.[7]

Genes associated with the age of menopause, and more specifically the length of the reproductive life span of a woman, are generally involved in DNA repair and maintenance as well as in the immune system.[8] In this way, genes involved in healthy ageing are also predictors for ovarian function. Indeed, a compromised ovarian function has been described in patients with type II diabetes mellitus[9] and in young girls with cancer.[10] This suggests that an unhealthy soma results in an early decline of ovarian function. It is unknown whether the same holds true for chronic inflammation.[8]

Rheumatoid arthritis (RA) is an example of a chronic inflammatory auto-immune disease that often affects women in their fertile age. RA not only affects the joints, but can also cause extra-articular damage, affecting different organ systems.[11,12] A prolonged time to pregnancy[13,14] and a younger age at which menopause sets in[15,16] are both indicators that in women with RA the ovarian function may be compromised as well.

In women with RA, AMH levels at time of diagnosis have been found to be comparable to those in healthy controls.[17] Furthermore, after 6 months of methotrexate (MTX) therapy in RA patients, AMH levels did not differ from those in patients who did not use MTX.[17] When analysing women with established RA, AMH levels were indeed lower compared to healthy controls.[18,19] However, thus far there have been no longitudinal studies on AMH levels in RA patients. Nor has the effect of disease characteristics on the decline of AMH levels over time been studied.

The objective of the current study was to investigate the intra-individual change in AMH levels over a longer time period in women with RA. We compared serum AMH levels in RA patients with those in controls from the general population, and studied the decline of serum AMH levels in RA patients over time in relation to RA-related clinical factors.

METHODS

Patients

For this observational cohort study, patients were recruited who had participated in the Pregnancy-induced amelioration of RA (PARA) study. The PARA study was a nationwide prospective cohort study, which was performed in the Netherlands in 2002-2010.[20] Patients were invited to participate by their attending rheumatologist if they had a diagnosis of RA according to the 1987 ACR criteria,[21] and if they were actively trying to conceive or were already pregnant. Patients had to have a good understanding of the Dutch language.[20] Patients could participate in the PARA study more than once. For the current follow-up study, patients were contacted when they had given consent to be contacted for future research, and when they had at least 1 non-pregnant serum sample available (i.e. from a preconception visit, or from a visit 6 months postpartum).

The majority of the PARA subjects had been contacted in 2013-2014 for a questionnaire on fertility.[22] As a result, addresses were up to date whenever possible. Patients currently living outside of the Netherlands or Belgium were excluded.

Ethical approval

The original PARA study, as well as the current follow-up study, were performed according to the Declaration of Helsinki, and were approved by the Medical Ethics Review Committee of the Erasmus MC.

Data collection

During the original PARA study,[20] patients were visited at their homes before, during, and after pregnancy, with the final visit 6 months postpartum. Each assessment consisted of a questionnaire driven interview on disease characteristics and use of medication, measurement of disease activity, and drawing of serum samples.
For the current study, patients received an information letter, and thereafter were contacted by telephone. Patients who gave informed consent, were visited at their home address for blood sampling, and they completed an online questionnaire including questions on menstrual cycle, age at menarche, hormone use, and other possible iatrogenic causes for amenorrhoea.

Measurements

Disease activity was measured during the PARA study visits using the Disease Activity Score assessing 28 joints (DAS28) for tenderness and swollenness, combined with serum C-reactive protein (CRP) levels.[23]
Serum samples were stored at -80°C. Serum AMH levels were measured in the samples from all preconception and 6 month postpartum PARA study assessments, as well as in the newly acquired samples from the follow-up visit.
AMH values were measured using the picoAMH assay, provided by Ansh labs (Houston, Texas, USA).[24,25] Inter and intra assay coefficients of variation were both < 5%.

Controls

Serum AMH levels in patients were compared to a group of healthy controls. This international reference cohort existed of 554 healthy adult women, aged 18-47 years, who had a regular menstrual cycle and/or were proven fertile.[26] AMH levels in controls were originally reported as measured by the Beckman Coulter Gen II Assay. Therefore,

a conversion factor was used to compare AMH levels in the patient group to those in the controls:[25]

$AMH\ (picoAMH\ assay\ in\ ng/mL) = (1.45 * AMH_{Gen\ II}) + 0.32$

Statistical analysis

Values are presented as mean ± SD for normally distributed variables, as median (IQR) if values were non-normally distributed, and as number (%) for dichotomous variables. The number of missing values are given for each variable.

To approach a normal distribution, AMH levels were log-transformed for analysis. To correct for different distribution of ages between patients and controls at the different time points, AMH levels were compared to controls using analysis of covariance (ANCOVA) on log-transformed AMH levels with adjustment for age.

Sensitivity analyses were performed by excluding women using combined oral contraceptives, and by excluding women using any steroid sex hormones.

To reduce potential bias in the longitudinal analysis due to missing values, multiple imputation was performed. To incorporate the longitudinal outcome into the imputation procedure, a preliminary linear mixed model (LMM) with a random intercept and random slope was fitted, using only completely observed covariates, and age as time variable. The random intercept and slope estimated by this model were considered a summary of the outcome, and were added as predictor variables to the imputation models.[27]

To study the effect of RA-related factors (disease duration, presence of ACPA, presence of RF, presence of erosions, past use of MTX) on the AMH levels, a LMM with random intercept and slope was built, using biological age as time variable. To obtain a more normal distribution of residuals and random effects in the LMM, a square root transformation was applied to the AMH levels. Interactions between ACPA, RF, and erosions, and between age and disease duration, were considered in the model. Pooled results from the 10 multiple imputed datasets are presented.

Since gynaecological age (i.e. years since menarche) may more precisely explain the inter-individual difference than biological age, a second analysis was performed using gynaecological age as time variable.

Statistical analysis was performed using Stata SE v14 (College Station, Texas, USA) and R version 3.3.1 (2016, the R foundation for statistical computing; packages used: mice, lme4).

RESULTS

Patients

Serum samples for 128 patients (43% of the original PARA cohort) were available for analysis. (Figure 1) For 2 patients no questionnaire data were available for the latest visit, but they were included in the analyses. Baseline characteristics are given in Table 1.

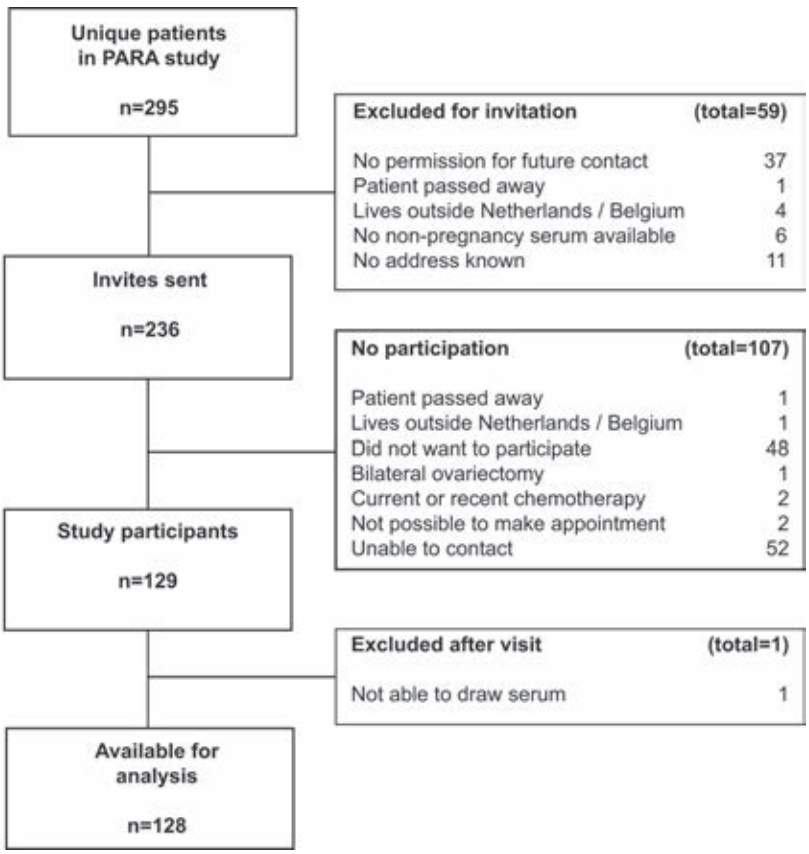

Figure 1 – Flow chart showing the patients from the original PARA study that did participate in the current study and were available for analysis

Table 1 – Characteristics of women with rheumatoid arthritis who participated in the PARA study and had a follow-up assessment in 2015/2016 (N=128)

Variables			Variables		
Age (years)			*Anti-rheumatic drugs at follow-up*		
	at first visit	31.8 ± 3.8	Methotrexate		65 (51%)
	at follow-up	42.6 ± 4.4	Prednisone		16 (13%)
			Sulfasalazine		15 (12%)
Age at menarche (years)		13.1 ± 1.4	Hydroxychloroquine		17 (13%)
	missing	9 (7.0%)	Leflunomide		5 (3.9%)
Smokers at first visit		9 (7.0%)	Biologicals - total n (%)		71 (55%)
	missing	2 (1.6%)		Etanercept	29 (23%)
				Infliximab	-
Disease characteristics				Certolizumab	2 (1.6%)
Duration of disease (years)				Adalimumab	20 (16%)
	at first visit	4.8 (2.1-10.4)		Golimumab	3 (2.3%)
	at follow-up	15.8 (12.7-21.5)		Abatacept	6 (4.7%)
	Missing	1 (0.8%)		Rituximab	2 (1.6%)
Presence of ACPA, n (%)		78 (61%)		Tocilizumab	9 (7.0%)
Presence of RF, n (%)		85 (66%)			
Presence of erosions, n (%)		77 (60%)	*Use of methotrexate*		
	missing	3 (2.3%)	Ever used MTX		113 (88%)
				missing	2 (1.6%)
Hormone use at follow-up			Cumulative duration of MTX use		
None		62 (48%)		never used	13 (10%)
Oral contraceptive pill		25 (20%)		less than 1 year	12 (9.3%)
Levonorgestrel releasing IUD		37 (29%)		1 to 5 years	27 (21%)
Injectable progesterone		2 (1.6%)		5 to 10 years	31 (24%)
	missing	2 (1.6%)		>10 years	25 (20%)
				missing	20 (16%)
			Cumulative years MTX in users		5.8 (2.2-10.2)

RA = rheumatoid arthritis ; ACPA = anti-citrullinated protein antibodies ; RF = rheumatoid factor ; IUD = intra-uterine device ; MTX = methotrexate

We compared the 128 study participants to the non-participating subjects (n=167) of the former PARA cohort (Table 2). There was a significant lower percentage of smokers among participants (7%) than in the non-participants (17%) (p=0.013). Furthermore, nulliparity was significantly more common among the non-participants (22%) than in the participants (4.7%) at the end of their final PARA episode (p<0.001). There were no significant differences in age, number of episodes participated in the PARA study, disease duration during the PARA study or current disease duration, presence of ACPA or RF, preconceptional disease activity during last PARA episode, or disease activity at the last PARA visit (either preconceptional or 6 months postpartum).

Of the participants, 41 (32%) women reported amenorrhoea (i.e. no menstruation in the preceding 12 months). In 29 women this could be explained by continuous oral contraceptive use (n=3) or the presence of a hormonal intra-uterine device (n=26), and 1 woman had a lactational amenorrhoea. Seven women had had a hysterectomy. In 4 women (3.1%) the amenorrhoea could not be explained by other causes, and they were considered postmenopausal. Their menopause had been at an age of 39, 44, 44 and 45 years respectively. Three of them had AMH values below the LoD, and in 1 patient the serum AMH level was 0.0114 ug/L.

Table 2 - Comparison of participants and non-participants in the follow-up of the PARA study

	Current study	Non-participants	P value
Number of unique patients	n=128	n=167	
Age[ϥ] - years	42.5 ± 4.4	43.4 ± 4.1	0.055[§]
Age at last PARA episode - years	32.0 ± 3.9	32.8 ± 3.9	0.11[§]
Smoker during PARA study	9 (7%)	28 (17%)	0.013[†]
missing	2 (2%)	4 (2%)	
Number of episodes within PARA study			
1 episode	98 (77%)	139 (83%)	
2 episodes	24 (19%)	24 (14%)	
3 episodes	6 (5%)	3 (2%)	
4 episodes	-	1 (1%)	
Obstetric history			
Never pregnant before PARA study	73 (57%)	93 (56%)	0.91[†]
Pregnant during last PARA episode	117 (91%)	120 (72%)	<0.001[†]
Nulliparous at end of PARA study	6 (4.7%)	36 (22%)	<0.001[†]
Disease Characteristics			
Duration of RA[ϥ] - years	15.6 (12.6 - 20.9)	15.5 (13.1 - 20.5)	0.88[‡]
missing	-	2 (1%)	
Duration of RA at first PARA episode	4.2 (2.0 - 9.6)	4.7 (1.9 - 8.5)	0.99[‡]
missing	-	2 (1%)	
Presence of ACPA	78 (61%)	112 (67%)	0.33[†]
Presence of RF	86 (67%)	125 (75%)	0.16[†]
Presence of erosions	77 (60%)	100 (60%)	0.61[†]
missing	3 (2.3%)	9 (6.4%)	
Disease activity Score (DAS28) during last PARA episode			
Preconception	3.6 ± 1.3	3.8 ± 1.2	0.17[§]
missing	51(40%)	54 (32%)	
First trimester	3.5 ± 1.2	3.6 ± 1.2	0.57[§]
missing	37 (29%)	70 (42%)	
last available DAS28 (non-pregnant)	3.5 ± 1.2	3.5 ± 1.2	0.79[§]
missing	-	4 (2%)	

† Fisher's exact test; ‡ Mann-Whitney U test; § Student's t test

ϥ at January 1st 2016

Hormone measurements

Serum AMH levels during follow-up were compared to AMH levels in healthy controls (figure 2). The mean age in controls was 30.6 ± 7.4 years, with a range of 18.0 – 46.8 years. The age range for patients at the follow-up visit was 31.6 – 53.5 years. Corrected for age, AMH levels in women with RA at the follow-up visit were significantly lower than those in controls (p<0.001).

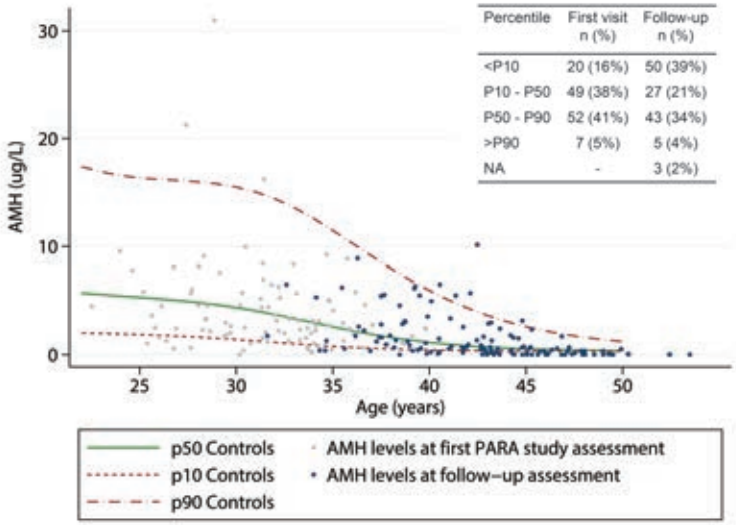

Figure 2 – First and last available serum anti-Müllerian hormone (AMH) levels (ug/L) in women with rheumatoid arthritis who participated in the Pregnancy-induced Amelioration of Rheumatoid Arthritis (PARA) study and were visited again after 6.4 – 13.7 years. The lines represent the 10th, 50th and 90th percentiles of AMH values in healthy controls (Lie Fong 2012)[26]

Looking at the age specific percentiles, 50 subjects (39%; 95%CI 31–48%) had a serum AMH level below the 10th percentile, compared to 20 patients (16%; 95%CI 9.3–22%) at the first available assessment (figure 2). At the follow-up assessment, 3 RA patients were older than 51 years, and as such, their age exceeded the age range of the nomogram (maximum age 51 years).

In patients not using oral contraceptives (n=100), the percentage of women with AMH levels below the 10th percentile was 36% (95%CI: 27–45%). In women not using any steroid sex hormones (n=61), this was 33% (95%CI 21–47%).

In Table 3 the age-specific AMH percentiles are shown for all subjects at the first and last measurement, grouped by disease characteristics. At the last assessment, higher percentages of patients with AMH levels below the 10th percentile were seen

in ACPA positive patients versus ACPA negative patients, in RF positive patients versus RF negative patients, and in patients with erosions at baseline versus those without erosions at baseline. However, these differences were not statistically significant.

Table 3 – Distribution of age-specific percentiles of serum AMH levels in women with rheumatoid arthritis from the PARA study, grouped by serology and erosions

	ACPA[$]		RF[$]		Erosions[$,ϑ]	
	neg	Pos	neg	pos	no	Yes
	(n=50)	(n=78)	(n=43)	(n=85)	(n=48)	(n=77)
First AMH measurement						
<P10	7 (14%)	13 (17%)	5 (12%)	15 (18%)	5 (10%)	15 (19%)
P10-P50	18 (36%)	31 (40%)	14 (33%)	35 (41%)	17 (35%)	32 (42%)
P50-P90	22 (44%)	30 (38%)	21 (49%)	31 (36%)	24 (50%)	26 (34%)
>P90	3 (6.0%)	4 (5.1%)	3 (7.0%)	4 (4.7%)	2 (4.2%)	4 (5.2%)
Last AMH measurement						
<P10	15 (30%)	35 (45%)	12 (28%)	38 (45%)	15 (31%)	34 (44%)
P10-P50	10 (20%)	17 (22%)	6 (14%)	21 (25%)	11 (23%)	16 (21%)
P50-P90	23 (46%)	20 (26%)	20 (47%)	23 (27%)	21 (44%)	20 (26%)
>P90	1 (2.0%)	4 (5.1%)	2 (4.7%)	3 (3.5%)	1 (2.1%)	4 (5.2%)
NA	1 (2.0%)	2 (2.6%)	3 (7.0%)	-	-	3 (3.9%)

Values are given as n(%)

ACPA = anti-citrullinated protein antibodies, RF = rheumatoid factor, AMH = anti-Müllerian hormone

$ at baseline

ϑ the presence or absence of erosions were missing for n=3 subjects

Linear mixed model

For 1 patient 8 measurements were available, 4 patients had 7 measurements, 1 patient had 6 measurements, 13 patients had 5 measurements, 50 patients had 4 measurements, 15 patients had 3 measurements, and 44 patients had 2 measurements. The intra-individual mean time between the first available AMH measurement and the last was 10.7 ± 1.8 years, with a range of 6.4 – 13.7 years. The mean age at the first AMH measurement was 31.8 ± 3.8 years, and at the final assessment 42.6 ± 4.4 years.

Repeated measurement analysis using a LMM showed a significant decrease of serum AMH levels with increasing age. None of the RA-related variables (disease duration, presence of ACPA, of RF, or of erosions, MTX use (ever)) did have a significant effect on the AMH levels over time in this group of 128 female RA patients (Table 4). Comparing

the full model shown in table 4 with a simple model of AMH based on age alone using a likelihood ratio test, did not reveal any significant differences, confirming the findings in table 4.

Table 4 - estimates of linear mixed model describing the change of serum anti-Müllerian hormone (AMH) levels (applying square root transformation) with increasing age in 128 female RA patients

Variable	Estimate	Standard error	95% Confidence interval			
(Intercept)	4.648	0.350	3.962	-	5.334	
Biological age - per year $	-0.077	0.007	-0.090	-	-0.063	ǂ
Disease duration ł	0.015	0.031	-0.046	-	0.076	
Presence of ACPA ł	-0.544	0.349	-1.227	-	0.140	
Presence of Erosions ł	-0.076	0.239	-0.545	-	0.393	
Presence of RF ł	-0.312	0.281	-0.863	-	0.239	
Ever used MTX	0.005	0.187	-0.361	-	0.372	
Age * Disease duration	-0.001	0.001	-0.002	-	0.001	
ACPA * Erosions	0.364	0.447	-0.512	-	1.239	
ACPA * RF	0.413	0.441	-0.451	-	1.276	
Erosions * RF	-0.146	0.390	-0.910	-	0.618	
ACPA* Erosions * RF	-0.121	0.573	-1.243	-	1.001	

ACPA = anti-citrullinated protein antibodies ; RF = rheumatoid factor ; MTX = methotrexate

$ age is used as time variable in the model

ł variable at baseline

ǂ p<0.05

The additional analysis with gynaecological age as time variable, was performed in the 122 patients that reported their age at menarche. Besides the decline of AMH with increasing gynaecological age, no other variables did show a significant effect. The full model based on gynaecological age was not significantly different from a simple model of AMH based on gynaecological age exclusively.

DISCUSSION

Women with RA had a compromised ovarian function compared to healthy controls, with a considerably larger proportion of women with RA having AMH levels below the age-specific 10th percentile. Assessment of serum AMH levels over time in female RA patients revealed that levels decreased more rapidly in patients compared to controls. Longitudinal analyses did not show any significant effect of RA-related factors on serum AMH levels.

The more rapid decline of serum AMH levels over time in women with established RA indicates that chronic inflammation compromises ovarian function. The latter may even decline faster than was currently found in women with RA since the participants in this study represented a more fertile selection, with also less smokers, than the original PARA cohort. Since the original PARA cohort was already a relative healthy cohort,[20] the true difference between women with RA and the general population may be even larger.

The compromised ovarian function in RA may be a direct effect of cumulative inflammatory damage to the ovaries. Although we found no significant association between disease activity and AMH levels in current or previous analyses, there is a relation with ACPA-positivity, which represents patients with on average more active disease, often requiring a more intensive treatment strategy.[28] We have previously reported significantly lower serum AMH levels in ACPA-positive patients,[19] and in the current study more ACPA-positive than ACPA-negative patients appeared to have AMH levels below the 10th percentile. Furthermore, in the linear mixed model, the negative association of ACPA-positivity with serum AMH levels was present, although non-significant, which may have been due to the lower number of subjects in this study. The present idea among rheumatologists is that ACPA positive RA may be a different disease than ACPA negative RA. It is not clear whether the reduced ovarian function is a result of this probably different disease mechanism, or of increased inflammatory damage due to longer periods of high disease activity. However, the lower AMH levels in patients who are ACPA-positive, fit with the overall concept that ACPA-positive RA is a more destructive disease with more extra-articular manifestations.[28]

Otherwise, there may be a genetic basis for the compromised ovarian function in RA. In genome-wide association studies (GWAS), not only genes linked to DNA repair and genome maintenance have been related to age at menopause, but also genes linked to immune response seem to determine the timing of menopause.[8] Whether RA and early menopause share a common genetic basis, may be the focus of future research.

Regarding generalizability, we should consider the recent changes in diagnosis and treatment of RA. Where patients from the PARA cohort were classified as having RA according to the 1987 ACR criteria,[21] nowadays RA is often recognized and diagnosed at an earlier stage, when less damage has been done.[29] Furthermore, new treatment guidelines have been developed over the last decade, focusing on early combination therapy in a treat-to-target regimen, and the addition of biological disease modifying antirheumatic drugs such as tumor necrosis factor (TNF) inhibitors for a tighter disease

management.[30] The next generation of young female RA patients may therefore have a milder course of the disease, with better long-term outcomes. Whether ovarian function and reproductive performance will also benefit from these changes, should be addressed in future studies.

Fifty percent of the current participants used hormonal contraceptives during the follow-up assessment, mainly progesterone releasing intra-uterine devices, or the oral contraceptive pill (OCP). The effect of exogenous steroid hormones on serum AMH levels is still unclear, with several studies reporting no significant effect of exogenous hormones on serum AMH levels, whereas other studies have shown, although subtle, lower AMH levels in users of either OCPs or progesterone only pills.[31,32] Sensitivity analyses excluding women using any of these hormones, still showed one-third of patients having AMH levels below the 10th percentile at follow-up. Therefore, the current use of contraceptives could not explain the decreased long-term serum AMH levels in our study group.

AMH levels in this study were measured with an ultrasensitive picoAMH assay. The comparison of AMH levels between patients and controls should be considered with caution, since the conversion factor applied to the controls has been developed in another laboratory than the laboratory that performed the measurements with the new assay. Inaccuracy due to the conversion, may give deviating results.[25] Furthermore, the currently applied picoAMH assay has a lower limit of detection compared to the Gen II assay that was used for the AMH measurements in controls. Nevertheless, adjusting the very low values in the current patients to the LoD applied in the controls, still resulted in a significantly higher percentage of patients with AMH values below the 10th percentile of controls.

In conclusion, serum AMH levels show a faster decline over time in women with RA compared to healthy controls, supporting the idea that in chronic inflammatory conditions, the body is less fit for reproduction. Optimal treatment of chronic inflammatory disease in an early phase may improve long-term women's health.

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Chapter 8

General discussion

This thesis illustrates the need for individualized decision making and a multidisciplinary approach in fertility care for patients with rheumatoid arthritis. Moreover, it provides some useful new insights into fertility, having distinct implications for both rheumatologists as well as for gynaecologists.

Implications for rheumatologists

In young women with rheumatoid arthritis who desire to build a family, for years the policy among rheumatologists has been to limit the prescription of antirheumatic drugs. Fear of possible teratogenic effects withheld intensive treatment in women during the preconception period.

Nowadays, attention for preconception care in patients with RA is gradually increasing among rheumatologists. In 2016, a EULAR task force has reported considerations on antirheumatic treatment in women before, during and after pregnancy.[1] However, concerns on preconception treatment are mainly limited to drug safety for the foetus during pregnancy and breastfeeding, whereas the effects on fertility are only briefly addressed.

This thesis has shown, that during the preconception period, antirheumatic treatment should be maintained, or adjusted in case teratogenic drugs are part of a patient's current treatment. Active disease should be treated intensively during the preconception period, both to reduce the negative effect of disease activity on the time to pregnancy, as well as to avoid the use of NSAIDs and high dosages of prednisone and their negative effect on fertility.

Developing adjusted treat-to-target guidelines for the preconception period can guide rheumatologists in effectively treating patients and minimalizing risks for pregnancy and offspring. Regarding fertility, treatment targets should preferably aim for disease remission to reduce time to pregnancy, with avoidance of the use of NSAIDs and high dosages of prednisone. The largest beneficial effect on time to pregnancy is to be expected in women with high disease activity, but patients with intermediate and low disease activity will also benefit. Previous studies on pregnancy and offspring in women with RA have already shown the importance of treating patients with active disease when they are planning a family. An increased maternal disease activity during pregnancy has been associated with lower birth weight[2] and rapid postnatal catch-up growth in the offspring, which in turn is associated with a higher risk for cardiovascular and metabolic disease in later life.[3] Furthermore, high and intermediate disease activity have both been associated with a higher occurrence of caesarian sections.[2] This thesis emphasizes the need for treatment adjustment early in the process of family planning.

Fertility care in a woman with rheumatoid arthritis is preferably started as soon as she is diagnosed. In all women of fertile age, rheumatologists should discuss family planning in an early stage. Patients should be made aware that their ovarian function may decline more rapidly compared to healthy women, especially when they are ACPA positive. In the light of the general tendency to postpone pregnancy beyond age 30 in Western countries, patients with ACPA positive disease especially should not delay trying to conceive. Whether the presence of ACPAs in serum by itself is responsible for the reduced ovarian function, or the more active disease associated with ACPA positivity[4] causes damage to the ovary in the long run, remains unclear. Follow-up of current early arthritis cohorts with implemented strict treat-to-target antirheumatic therapy, could clarify whether tight disease control has a protective effect on the ovarian function.

Besides general lifestyle recommendations regarding fertility, such as cessation of smoking, body weight reduction in obese patients, and the use of folic acid, sexual functioning should be a recurrent topic. The help of a sexologist should be offered when patients experience problems in this area.

Early consultation of a gynaecologist or endocrinologist to exclude other issues regarding fertility in the patient or her partner may prevent unnecessary delay of conception.

Implications for gynaecologists

Gynaecologists and fertility specialists attending patients with RA should be aware of the possible effects of the disease, its manifestations, and antirheumatic treatment on both fertility, as well as on different treatments. When active disease is present, it may be worthwhile to postpone fertility treatment until antirheumatic treatment has been optimized and resulted in less active disease or preferably remission, and the patient does not require regular NSAIDs use or high dosages of prednisone. The increased chance of spontaneous conception when the disease is more strictly controlled, should be considered and discussed with patients, thus avoiding possibly unnecessary fertility treatments.

The distinction between different rheumatic diseases should be made. Many rheumatic conditions are known, but in only a few the effect on fertility has been documented. This thesis demonstrates a reduced fertility and a decline in ovarian function in women with RA. However, in patients with systemic lupus erythematosus (SLE), fertility is not compromised, although miscarriages and pregnancy complications are more frequent, resulting in smaller family sizes in women with SLE.[5]

Studies on ovarian function in SLE show conflicting results.[6,7] In Sjögren's disease, no reduced fertility has been reported,[8,9] but lower AMH levels have been found.[10] Since the spectrum of rheumatic diseases is broad, collaboration between gynaecologists and rheumatologists is an essential part of preconception care in women with rheumatic diseases. Not only should the effect and safety of medical treatment in the preconception period be considered, but also the timing of fertility treatments. This will optimize the chance of a successful outcome.

Not only in patients with rheumatic diseases, but in all patients visiting a reproductive medicine unit, comorbidities and medication use should be considered as possible fertility compromising factors. Current guidelines on fertility assessments, such as the national guideline on fertility assessments of the Dutch Society of Obstetrics and Gynaecology (NVOG), and the guideline on 'Fertility Problems: Assessment and Treatment' by the National Institute for Health and Care Excellence (NICE) do mention possible effects of prescription and over-the-counter drugs on conception and pregnancy. However, comorbidity is only mentioned in the context of possible tubal occlusion and anovulation. Guidelines should also mention the possibility of direct interaction of comorbidity and fertility. For a patient with comorbidity, consultation of attending physicians on the effects of the condition on fertility and vice versa, and on possible treatment adjustments, as well as discussing timing of the start of fertility treatments, may both increase the chance of a positive outcome of fertility treatment as well as favour maternal health.

Suggestions for future research

The studies in this thesis have revealed several associations of rheumatoid arthritis and antirheumatic drugs with subfertility or a prolonged time to pregnancy. Prospective studies in women with RA who will start planning a family, may elucidate whether a strict control of the disease, and avoidance of preconception use of NSAIDs and high dosages of prednisone will result in less subfertility in this patient group. However, the effect of earlier diagnosis since the introduction of the ACR/EULAR 2010 classification criteria for rheumatoid arthritis,[11] and of the current treat-to-target guidelines, including use of biologic DMARDs[12] should be taken into consideration.

Active disease as well as the use of NSAIDs in women with RA, are associated with a longer time to pregnancy and subfertility. Hence, pain as a manifestation of active disease may be an underlying cause for a longer time to pregnancy, causing problems in sexual intercourse, or a reduced intercourse frequency. Since the studies in this thesis were post-hoc analyses of the PARA study, no information was available on intercourse

frequency and timing of intercourse during the menstrual cycle. Prospective studies in women with RA, documenting menstrual cycles and intercourse frequencies, along with pain scores and the use of antirheumatic drugs, may provide insight in the effect of pain and antirheumatic medication on sexual intercourse.

The mechanism behind subfertility in active disease may be subject of future research. Although the implantation rate after IVF or ICSI treatments in RA patients was high (chapter 3), the number of patients was low, and possible selection bias was present. Therefore, the possibility of failure of embryo implantation as underlying cause for subfertility should be considered. Many growth factors and cytokines are involved in the embryo-endometrium interaction.[13] Serum cytokine levels have been shown to be altered in RA patients. Exploratory studies on the association of serum cytokine levels in RA with subfertility or time to pregnancy may point towards cytokines of interest. Whether the intrauterine environment also suffers from this imbalance, might be studied subsequently by proteome studies in aspirated endometrial fluid. Identification of fertility impairing cytokines in the future may even indicate treatment targets in this specific patient group.

Regarding ovarian function, long term studies of serum AMH levels at different time points in existing cohorts of women with established RA, and studies in early arthritis cohorts, may provide information on the actual age at menopause. Studies on serum AMH levels in women receiving the current more intensive treatment strategies, can reveal whether early diagnosis and start of treatment, current treat-to-target guidelines, and treatment with biological DMARDs have a protective effect on ovarian function. AMH recovery has been described in childhood cancer survivors, in whom decreased AMH levels are found as well at diagnosis as shortly after chemotherapy. Future studies of serum AMH levels in RA, should reveal whether intensive treat-to-target antirheumatic treatment may lead to recovery of ovarian function in young women with RA.

Conclusion

The results in this thesis emphasize the need for personalized medicine in fertility care. Current national and international guidelines, both in rheumatology, as well as in reproductive medicine, should take into account the interaction between comorbidity and fertility. For patients with RA, but also for patients with other comorbidities, when planning a family, a multidisciplinary approach may limit the time to pregnancy and favour maternal health.

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Summary

Rheumatoid arthritis (RA) is one of the most common rheumatic diseases. Impaired fertility in women with RA has been reported, but little is known about the exact mechanisms behind subfertility in this patient group. The aims of this thesis were (I) to assess the time to pregnancy in a cohort of women with RA who wished to conceive, and to determine its association with clinical aspects of RA, (II) to study the occurrence of miscarriages in RA, and (III) to research the ovarian function in RA, both during the preconception period, as well as (IV) during a longer time period.

The introduction in **chapter 1** gives a general overview of RA, its diagnosis and treatment. Furthermore, a description of ovarian function, and the use of anti-Müllerian hormone (AMH) as a serum marker for this ovarian function is given. After a brief explanation on subfertility in the general population, an overview is given on what is known about fertility problems, miscarriages, and menopause in women with RA. Finally, the design of the Pregnancy Induced Amelioration of RA (PARA) study is described, since the studies in this thesis were performed mainly within the PARA cohort.

Chapter 2 describes the increased time to pregnancy (TTP) within the PARA cohort and its relation with several disease characteristics and anti-rheumatic treatment. A longer TTP was related to age, nulliparity, disease activity (DAS28), and preconception use of non-steroidal anti-inflammatory drugs (NSAIDs) and prednisone. The effect of prednisone was dose-dependent, with a longer TTP when higher dosages of prednisone were taken.

The outcomes of fertility assessments in RA patients are addressed in **chapter 3**. After fertility work-up, subfertile women with RA often received a diagnosis of unexplained subfertility, or of anovulation. Unexplained subfertility was related to periconception use of NSAIDs, suggesting that these drugs have a negative effect on fertility. A relative high percentage of pregnancies were the result of fertility treatments. Despite the higher incidence of subfertility in the study group, the outcome of fertility treatments appeared favorable.

In **chapter 4**, the association between miscarriages and RA related clinical factors is studied. The miscarriage rate in the PARA cohort was comparable to that in the general population. There was a possible association of the occurrence of miscarriages with ACPA positive disease, but this was not statistically significant. The majority of patients who had miscarried and continued trying to conceive, achieved an ongoing, successful pregnancy within one year after their miscarriage.

The ovarian function in early RA is evaluated in **chapter 5**. Serum AMH levels in women within the Rotterdam Early Arthritis Cohort who had recently been diagnosed with RA were not different from AMH levels in healthy controls. There was no association of AMH levels with RA serology, the presence of erosions, or disease activity. Furthermore, six months of methotrexate therapy did not result in a faster decline of serum AMH levels.

Chapter 6 returns to the PARA cohort, studying the ovarian function in the preconception period and its relation with time to pregnancy. In this group of women with a median disease duration of RA of 4.5 years, serum AMH levels were significantly lower than in healthy controls, with 17% of patients below the age-specific 10th percentile of healthy controls. Serum AMH levels were lower in ACPA positive patients. Time to pregnancy was not associated with AMH levels.

The decline of ovarian function in RA over a longer period is studied in **chapter 7**. For this purpose a follow-up study of the women participating in the PARA-cohort was undertaken, with last serum samples being taken at a median disease duration of 15.8 years. Over the years, serum AMH levels in women with RA showed a steeper decline than healthy controls with 39% of women having serum AMH levels below the 10th percentile of healthy controls at the last observation.

Finally, in the general discussion in **chapter 8** clinical implications for both rheumatologists and gynaecologists are described, and suggestions for future research are given. The importance of individualized medicine in women with rheumatoid arthritis is discussed.

Samenvatting

Reumatoïde artritis (RA) is een van de meest voorkomende reumatische aandoeningen. Uit eerdere onderzoeken blijkt dat vrouwen met RA verminderd vruchtbaar zijn, maar welk mechanisme hieraan ten grondslag ligt is niet bekend. Dit proefschrift had als doel het bestuderen van (I) de duur tot het tot stand komen van een zwangerschap bij vrouwen met RA en het verband daarvan met ziektegerelateerde factoren, (II) het optreden van miskramen bij vrouwen met RA, (III) de ovariële functie van vrouwen met RA in de preconceptionele periode en (IV) de ovariële functie op de lange termijn.

De inleiding in **hoofdstuk 1** geeft een algemeen overzicht van de ziekte RA, de diagnostiek, en de behandeling. Vervolgens wordt uitleg gegeven over de ovariële functie en het gebruik van anti-Müllers hormoon (AMH) in het serum als indicatie voor deze ovariële functie. Na een korte toelichting over subfertiliteit in de algemene bevolking, wordt beschreven wat al bekend is over vruchtbaarheid, miskramen en menopause in vrouwen met RA. Tot slot wordt de PARA-studie beschreven, welke de basis is geweest voor een groot deel van dit proefschrift.
Hoofdstuk 2 beschrijft de langere duur tot zwangerschap binnen het PARA-cohort en de relatie hiervan met verschillende ziekteaspecten en anti-reumatische therapie. Een langere tijd tot zwangerschap was gerelateerd aan leeftijd, nulliparitеit, ziekteactiviteit en preconceptioneel gebruik van non-steroidale anti-inflammatoire middelen (NSAID's) en prednison. Het effect van prednison was dosis-afhankelijk, waarbij het langer duurde tot een vrouw zwanger werd wanneer zij een hogere dosering prednison gebruikte.
De uitkomsten van vruchtbaarheidsonderzoek bij vrouwen met RA worden beschreven in **hoofdstuk 3**. Na oriënterend fertiliteitsonderzoek kregen vrouwen met RA vaker de diagnoses 'onverklaarde subfertiliteit' en 'anovulatie'. Vrouwen die voor of rondom de conceptie NSAID's gebruikten, waren vaker onverklaard subfertiel, wat suggereert dat deze middelen een negatieve invloed hebben op de vruchtbaarheid. Verder was een relatief hoog percentage zwangerschappen het resultaat van vruchtbaarheids-behandelingen. Ondanks het hogere percentage vrouwen met subfertiliteit in de studiegroep, lijken de uitkomsten van vruchtbaarheidsbehandelingen gunstig.
In **hoofdstuk 4** wordt het verband tussen miskramen en RA-gerelateerde klinische factoren onderzocht. Miskramen traden bij de zwangere vrouwen in het PARA-cohort even vaak op als in de algemene bevolking. Er was mogelijk een relatie tussen het optreden van een miskraam en ACPA-positieve ziekte. Het merendeel van de patiënten die na een miskraam opnieuw probeerden zwanger te worden, bereikte dit binnen een jaar met een levend geboren kind als resultaat.

De ovariële functie bij vrouwen met recent gediagnostiseerde RA is onderzocht in **hoofdstuk 5**. De serumwaarden van het AMH ten tijde van de diagnose RA verschilden niet van die van gezonde controlevrouwen. Er was geen verband tussen de hoogte van het AMH en serologie, de aanwezigheid van erosies, of ziekteactiviteit. Ook bleek zes maanden behandeling met methotrexaat geen snellere daling van AMH-waarden te veroorzaken.

In **hoofdstuk 6** wordt in het PARA-cohort de ovariële functie tijdens de periode voor de bevruchting bekeken, en de relatie met de tijd tot zwangerschap. Van deze groep vrouwen met een mediane ziekteduur van 4.5 jaar had 17% AMH-waardes onder het 10^{e} percentiel van gezonde controles. Lagere AMH-waarden werden gevonden in ACPA-positieve patiënten. De tijd tot zwangerschap had geen verband met de hoogte van het AMH.

De afname van de ovariële functie over een langere periode wordt onderzocht in **hoofdstuk 7**. Hiervoor werden vrouwen uit het PARA-cohort opnieuw onderzocht bij een mediane ziekteduur van 15.8 jaar. Bij deze vrouwen met RA werd over de tijd een sterkere afname van AMH-waarden gezien dan in gezonde controles. Bij de laatste observatie, had 39% van de vrouwen met RA een AMH-waarde onder de 10^{e} percentiellijn van de gezonde controles.

Tot slot wordt in de algemene discussie in **hoofdstuk 8** besproken welke gevolgen de resultaten in dit proefschrift hebben voor de klinische praktijk, zowel voor de reumatologen als de gynaecologen. Ook worden suggesties gegeven voor toekomstig onderzoek. Het belang van een individuele, patiëntgerichte benadering in de zorg voor vrouwen met RA met een kinderwens wordt besproken.

Addendum

Authors and Affiliations

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Visser JA	Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, the Netherlands

List of abbreviations

ACPA	anti-citrullinated protein antibody
ACR	American College for Rheumatology
AMH	anti-Müllerian hormone
ANCOVA	analysis of covariance
anti-CCP	anti-cyclic citrullinated peptide
BMI	body mass index
CI	confidence interval
COX	cyclo-oxigenase
CRP	C-reactive protein
DAS	disease activity score
DAS28	disease activity score with a 28 joint count
DAS28-CRP	DAS28 including CRP
DAS28-CRP-3	DAS28 including CRP and excluding global health measurement
DMARD	disease-modifying anti-rheumatic drug
DNA	desoxyribonucleic acid
EliA	enzyme linked immunoabsorbent assay
ELISA	enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FSH	follicle stimulating hormone
FT	fertility treatment
GH	global health
GWAS	genome-wide association studies
HR	hazard ratio
ICSI	intra-cytoplasmic sperm injection
IgM	immunoglobulin M
IQR	interquartile range
IUD	intra-uterine device
IUI	intra-uterine insemination
IVF	in vitro fertilization
LMM	linear mixed model
LoD	limit of detection
LUF	luteinized unruptured follicle
MOH	mild ovarian hyperstimulation

MTX	methotrexate
NICE	National Institute of Health and Care Excellence
NSAID	non-steroidal anti-inflammatory drug
NVOG	Nederlandse Vereniging voor Obstetrie en Gynaecologie
OCP	oral contraceptive pill
OI	ovulation induction
OR	odds ratio
P10	10th percentile
P50	50th percentile
p90	90th percentile
PARA	pregnancy-induced amelioration of rheumatoid arthritis
PI	prediction interval
POI	premature ovarian insufficiency
RA	rheumatoid arthritis
REACH	Rotterdam early arthritis cohort
RF	rheumatoid factor
SD	standard deviation
SLE	systemic lupus erythematosus
TGF	transforming growth factor
TNF	tumor necrosis factor
TTP	time to pregnancy
VAS	visual analogue scale
WHO	World Health Organization

Over de auteur

Jenny Brouwer werd geboren op 28 november 1983 in Den Helder. Zij groeide samen met haar broer en twee zussen op in Anna Paulowna en haalde in 2002 haar gymnasiumdiploma cum laude aan het Etty Hillesum College te Den Helder. Vervolgens startte zij de opleiding Geneeskunde aan de Vrije Universiteit te Amsterdam. Haar wetenschappelijke stage liep zij in 2005-2006 aan de subafdeling Voortplantings-endocrinologie van het VU medisch centrum, onder supervisie van dr. C.B. Lambalk. Zij onderzocht hierbij het voorkomen van verhoogde serumwaarden van het luteïniserend hormoon bij vrouwen met polycysteus ovariumsyndroom.

Na het cum laude behalen van het artsexamen in 2008 begon zij als fertiliteitsarts aan de subafdeling Voortplantingsgeneeskunde van de afdeling Verloskunde en Gynaecologie in het Erasmus MC te Rotterdam. In 2011 is zij naast haar klinische werkzaamheden gestart aan het onderzoeksproject over vruchtbaarheid van vrouwen met reumatoïde artritis, een samenwerkingsverband van de afdeling Reumatologie en de subafdeling Voortplantingsgeneeskunde van de afdeling Verloskunde en Gynaecologie. Onder supervisie van prof. dr. J.M.W. Hazes, prof. dr. J.S.E. Laven en dr. R.J.E.M. Dolhain mondde dit uit in een volledig promotietraject en in dit proefschrift.

Tijdens haar onderzoeksperiode volgde Jenny tevens de master Clinical Epidemiology aan de Netherlands Institute of Health Sciences (NIHES), welke zij in 2016 afrondde. Daarnaast is zij sinds 2014 bestuurslid van de Vereniging van Fertiliteitsartsen (VVF).

Jenny woont samen met Sjel Saltzherr. Samen zijn zij trotse ouders van zoons Robin (2013) en Thomas (2017).

About the author

Jenny Brouwer was born on November 28th 1983 in Den Helder, and grew up in Anna Paulowna. In 2002, she finished her pre-university education at the Etty Hillesum College in Den Helder. She studied medicine at the Vrije Universiteit in Amsterdam, and obtained her medical degree in 2008.
Next, she started as a physician at the Fertility Center of the division of Reproductive Medicine, department of Obstetrics and Gynaecology, at the Erasmus MC in Rotterdam. In addition to her clinical work, she started as a PhD student on the research project on fertility in women with rheumatoid arthritis, under supervision of prof. dr. J.M.W. Hazes (Rheumatology), prof. dr. J.S.E. Laven (Reproductive Medicine), and dr. R.J.E.M. Dolhain (Rheumatology).

During her PhD period, Jenny achieved a Master's degree in Clinical Epidemiology at the Netherlands Institute of Health Sciences (NIHES) (2016). Since 2014, she has been a board member of the Dutch society for physicians in reproductive medicine (VVF).

Jenny lives with Sjel Saltzherr. Together they have two sons, Robin (2013) and Thomas (2017).

List of publications

Related to this thesis

Brouwer J, Laven JSE, Hazes JMW, Schipper I, Dolhain RJEM. **Levels of serum anti-Mullerian hormone, a marker for ovarian reserve, in women with rheumatoid arthritis.** Arthritis Care Res (Hoboken). 2013 Apr 1;65(9):1534-8.

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Not related to this thesis

Hendriks ML, Brouwer J, Hompes PG, Homburg R, Lambalk CB. **LH as a diagnostic criterion for polycystic ovary syndrome in patients with WHO II oligo/amenorrhoea.** Reprod Biomed Online. 2008 Jun;16(6):765-71.

PhD Portfolio

Summary of PhD training and teaching

Name PhD student: J. Brouwer
Erasmus MC Departments:
- Rheumatology
- Obstetrics and Gynaecology - division of Reproductive Medicine

Research School: NIHES

PhD period: 2011 - 2016
Promotors:
- Prof. dr. J.M.W. Hazes
- Prof. dr. J.S.E. Laven

Co-promotor:
- Dr. R.J.E.M. Dolhain

PhD training

	Year	ECTS
General courses		
- Biomedical English Writing and Communication	2013	4.0
- BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2013	1.0
- Integrity in Science	2016	0.3
Specific courses (e.g. Research school, Medical Training)		
- Principles of Research in Medicine (NIHES)	2011	0.7
- Biostatistics for Clinicians (NIHES)	2012	1.0
- Regression Analysis (NIHES)	2012	1.9
- Logistic Regression (NIHES)	2012	1.4
- NIHES master in health sciences – specialization Clinical Epidemiology	2013-2016	70
Seminars and workshops		
- Workshop on Photoshop and Illustrator CS5 for PhD-students and other researchers (MolMed)	2012	0.3
- Workshop on InDesign CS5 for PhD-students and other researchers (MolMed)	2012	0.2
- Erasmus MC PhD day	2014	0.2

Presentations

-	Najaarsdagen, Nederlandse Vereniging van Reumatologie (NVR), annual scientific meeting Arnhem, NL. *Poster presentation*	2012	1.0
-	American College of Rheumatology (ACR), annual meeting Washington, USA. *Poster presentation*	2012	1.0
-	European League Against Rheumatism (EULAR) annual meeting, Madrid, Spain. *Poster presentation*	2013	1.0
-	Najaarsdagen, Nederlandse Vereniging van Reumatologie (NVR), annual scientific meeting Arnhem, Nl. *Oral presentation*	2013	1.0
-	American College of Rheumatology (ACR) annual meeting, San Diego, USA. *Oral presentation*	2013	1.0
-	Wladimiroff Symposium, Erasmus MC. *Oral presentation*	2014	1.0
-	VIIIth International Conference on Pregnancy and Rheumatic Diseases, Trondheim, Norway. *Oral presentation*	2014	1.0
-	American College of Rheumatology (ACR) annual meeting, Boston, USA. *Poster presentation*	2014	1.0
-	European League Against Rheumatism (EULAR) annual meeting, Rome, Italy. *Oral presentation*	2015	1.0
-	European Society for Human Reproduction and Endocrinology (ESHRE) annual meeting, Lisbon, Portugal. *Oral presentation*	2015	1.0
-	Najaarsdagen, Nederlandse Vereniging van Reumatologie (NVR), annual scientific meeting, Arnhem, NL. *Poster presentation*	2015	1.0
-	Vereniging van Fertiliteitsartsen (VVF), jaarlijkse ESHRE avond, Utrecht, NL. *Oral presentation*	2015	0.5
-	IXth International Conference of Reproduction, Pregnancy, and Rheumatic Diseases, San Diego, USA. *Oral presentation*	2016	1.0
-	European League Against Rheumatism (EULAR) annual meeting, Madrid, Spain. *Poster presentation (2x)*	2017	1.0
-	European Society for Human Reproduction and Endocrinology (ESHRE) annual meeting, Geneva, Switzerland. *Poster presentation*	2017	1.0

Teaching activities

	Year	ECTS
Lecturing		
- Regionale Opleidingsdag AIOS Reumatologie, Rotterdam, NL, *oral presentation*; 'Vrouwelijke subfertiliteit in reumatoïde artritis'.	2013	1.0
Supervising practicals and excursions, Tutoring		
- Teaching 3rd year medical students (Reproductive Medicine), Erasmus MC	2011-2017	5.0
- Teaching 4th year medical students (Epidemiology), Erasmus MC	2015	0.5
Supervising Master's theses		
- Supervising elective research program (21 weeks) of 4th year medical student R. Fleurbaaij	2014	1.5

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