The Impact of Rheumatoid Arthritis during Pregnancy on Mother and Child

Hilal Ince-Aşkan

The Impact of Rheumatoid Arthritis during Pregnancy on Mother and Child

De impact van reumatoïde artritis tijdens de zwangerschap op moeder en kind.

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Hilal Ince-Aşkan

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Science is the most
reliable guide for civilization,
for life, for success
in the world *(M.K. Atatürk)*

Voor mijn ouders

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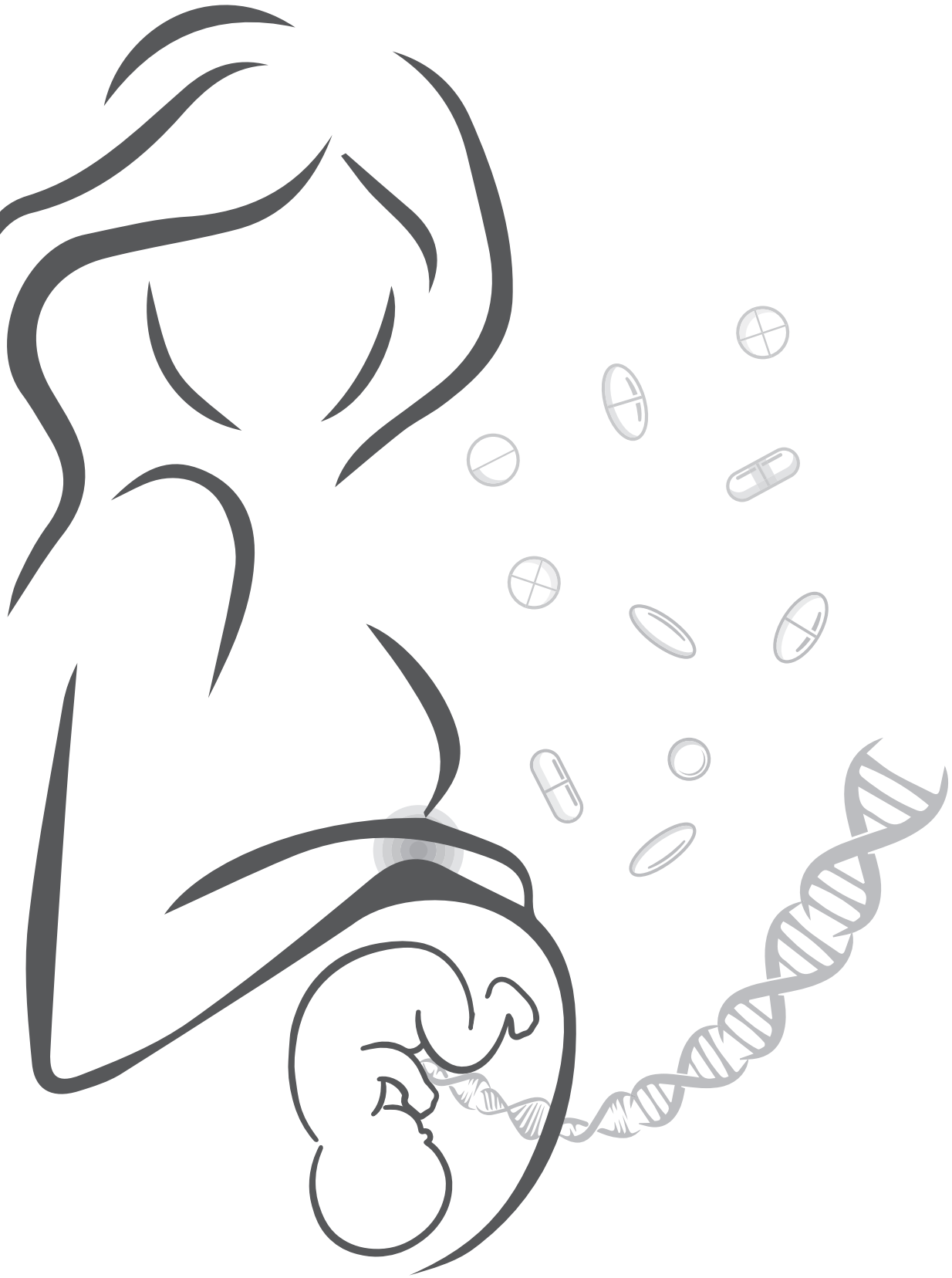


General introduction

1

Rheumatoid arthritis (RA) is a quite common autoimmune disease, also affecting pregnant women. The impact of RA during pregnancy on mother and child is largely unknown. This introduction will first provide knowledge on RA in general, around pregnancy and during lactation. Next, a description will be given on the developmental origins of health and disease (DOHaD) hypothesis, which contributes to the explanation of the impact of RA during pregnancy on the child. Furthermore, the role of cortisol and epigenetics (in particular DNA methylation) within this hypothesis will be summarized. This will be followed by a description of the designs of the studies. Finally, the aims of this thesis will be described as well as its outline.

1.1 RHEUMATOID ARTHRITIS

RA is a disease characterized by a chronic, systemic inflammation and the presence of the autoantibodies[1], affecting synovial membranes of joints, which can lead to significant joint destruction[2]. The prevalence of RA ranges from 0.5 to 2%, mostly affecting women between 40 and 50 years[2], yet also often during fertile age[3].

It is being thought that the development of RA is a complex interaction between a high-risk genetic profile in combination with environmental exposures (e.g. cigarette smoking[4]) and epigenetic changes[5]. Although RA is predominantly an articular disease, approximately 50% of the patients develop some kind of "extra articular manifestations" (EAMs) associated with the disease[2, 6]. EAMs include involvement of the skin, eyes, cardiovascular system, lungs, and nervous system[2]. The presence of the RA related autoantibodies rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) is associated with more progressive joint damage and more severe EAM[7].

1.2 PREGNANCY AND RA

1.2.1 Fertility

Multiple studies have shown that fertility in female patients with RA is impaired[8-12]. Women with RA have a prolonged time to pregnancy (TTP), have a smaller family size and are more likely to receive fertility treatment[12, 13]. In the past, it had been hypothesized that impaired fertility in RA is due to a smaller ovarian reserve[14], which might already be present before diagnosis. At diagnosis, the levels of anti-Müllerian hormone (AMH), a biomarker for ovarian reserve, are not different in premenopausal in women with RA versus healthy controls[15]. However, AMH levels in patients with RA decline more rapidly over time compared to healthy controls, indicating that RA might have a negative impact on the ovarian reserve[16].

A longer TTP in female patients with RA has been associated with older age, nulliparity, higher RA disease activity, and the use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and prednisone (>7.5 mg/day) before conception[12].

1.2.2 The effect of pregnancy on RA

In 1938, Hench[17] was the first who described the spontaneous improvement of RA during pregnancy and the flare postpartum. Since then, research on pregnancy and RA increased in popularity, however there are still limited numbers of prospective cohort studies within this field of research. Multiple retrospective[8, 18-25] and some prospective studies[26-31] reported the pregnancy related improvement of RA disease activity and the postpartum flare. Initially, higher improvement rates around 90% were reported, but in the more recent prospective studies these rates were around 50%[32]. Discrepancies between studies are probably caused by differences in study design, patient selection, and definitions of improvement[32]. Especially patients who do not express the autoantibodies RF and ACPA are more likely to improve during pregnancy[33]. Although RA disease activity decreases in approximately half of the patients, the mean improvement is limited, and only a small number of patients achieves remission during pregnancy[30, 31].

The pregnancy-associated improvement of RA and the flare of the disease activity postpartum is thought to be the result of multiple immunological and hormonal changes[34]. One of the theories behind this phenomenon is the shift of T helper (Th) cells from a pro-inflammatory Th1 to an anti-inflammatory Th2 profile during pregnancy[35]. Another theory is the influence of female sex hormones on RA disease activity, since pregnancy is associated with changes in estrogen and progesterone levels[36, 37], RA is more prevalent in women compared to men[38], and oral contraceptives have a protective effect on the onset of rheumatoid arthritis[39].

1.2.3 The effect of RA on pregnancy and outcome

RA is thought to impair pregnancy outcome, although not to that extend that has e.g. been described for Systemic Lupus Erythematosus(SLE)[40].

Some studies[41, 42], but not all[43], have shown that the risk of preeclampsia is slightly increased in RA. In addition, women with RA have an increased risk of a cesarean section, especially if the disease activity is higher[32, 43]. Furthermore, the risk of preterm delivery is increased in female patients with RA[41, 42], especially if they have higher RA disease activity during pregnancy[44]. Also, the use of prednisone during pregnancy is associated with a shorter gestational age[43].

Fortunately, female patients with RA do not have an increased risk of offsprings with congenital malformations or perinatal death[41, 45], and the risk of a miscarriage is comparable with the general population[46].

Children born to women with higher RA disease activity have a lower birth weight, although still within the normal range, even when correcting for gestational age[43, 47]. Those children also show rapid catch-up growth in weight, defined as a weight gain standard deviation score (SDS) of >0.5 during the first 3 months in infants with a weight gain of >0.67 SDS in the first year of their life[43, 47]. The effect of RA on pregnancy outcome might have lifelong consequences for the offspring, since a lower birth weight in combination with rapid catch-up growth in weight is associated with future metabolic and cardiovascular disease[48, 49].

1.2.4 Lactation and RA

In the World Health Organization's (WHO's) infant feeding recommendation it is advised that infants should be exclusively breastfed for the first 6 months of life in order to achieve optimal growth, development and health. After those 6 months, breastfeeding should be continued for up to two years of age or beyond[50]. These recommendations are based on the fact that breast milk, which is a completely personalized nutrition source, has multiple beneficial effects for mother and child[51]. Breastfed children have a lower risk of Sudden Infant Death Syndrome(SIDS)[52], infectious diseases[53], diabetes[53], obesity, high cholesterol or high blood pressure in adulthood[54, 55]. In addition, breast milk might be able to modulate imprinting events during the first months of life in terms of development of the immune system and susceptibility to mainly immune-mediated diseases, resulting in long-term effects for the offspring[51]. Besides the numerous benefits in general, breastfeeding is associated with additional beneficial effects for premature infants in terms of a decreased risk of sepsis, meningitis and retinopathy, and improved neurodevelopmental outcomes[53].

There have not been many studies on lactation in female patients with RA. It's not known if women with RA breastfeed their offsprings more or less often compared to healthy mothers. In addition, only one prospective study[56] has focused on the postpartum flare in relation with lactation. They showed that first-time breastfeeding RA patients had higher disease activity within 6 months postpartum, even after correcting for treatment, compared to patients with RA who had breastfed before and patients with RA who were not breastfeeding[56]. Given the previous conflicting results on breastfeeding and the onset of RA, more studies are required to draw any firm conclusions on the relationship between breastfeeding and the risk of a postpartum flare.

1.2.5 Treatment of RA during pregnancy and lactation

Treatment of RA during pregnancy is challenging. On one hand disease activity needs to be suppressed in order to improve health and to achieve better outcomes for mother and child. On the other hand medication with possible harmful side effects for the offspring, like methotrexate and leflunomide, must be avoided during pregnancy[57-59]. More knowledge

on long-term effects of high disease activity and medication use is necessary in order to find a balance between accepting higher disease activity versus intensifying treatment.

Until the first decade of this century it was assumed that RA improved during pregnancy, therefore and because of fear for side effects, rheumatologist mostly tried to avoid medication as much as possible, but when this was not feasible, treatment was restricted to sulfasalazine, prednisone and hydroxychloroquine[57].

The use of Tumor Necrosis Factor (TNF) inhibitors in RA has increased in the last years, also during pregnancy. Most safety data are on the use of the TNF inhibitors infliximab and adalimumab during early pregnancy. Use during first and second trimester is not associated with an increased risk of miscarriages or congenital malformations[60].

At the time the study in this thesis was performed, the use of hydroxychloroquine, sulfasalazine, azathioprine, prednisone, and most NSAIDs were considered compatible with lactation[57].

1.3 THE DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

Environmental conditions during early life, such as before conception, during pregnancy and in infancy may have lifelong consequences[61, 62]. According to the DOHaD hypothesis, first described by Barker[63], after intrauterine growth restriction due to (relative) undernourishment during pregnancy, either direct (e.g. maternal malnutrition) or indirect through placental malfunction (e.g. maternal disease), a child is biologically programmed to improve its chances of survival on a low caloric intake[48, 64]. If, however, after birth the nutrient availability is abundant[64], the child will show rapid catch-up growth for weight[49]. The combination of lower birth weight with a rapid catch-up growth for weight is associated with an impaired glucose tolerance[64], a higher fat percentage[49] and therefore an increased risk for cardiovascular[65] and metabolic diseases[66] in adulthood. Fetal growth restriction and adult onset disease are thought to be linked by epigenetic processes[64, 67, 68]. The long-term consequences of a lower birth weight are irrespective of the cause of this lower birth weight.

In the Dutch Hunger Winter study, exposure to famine during pregnancy and its long-term health consequences on the offspring have been explored[69, 70]. In this study, exposure to famine during any stage of gestation was associated with glucose intolerance[70, 71]. Women who were undernourished during mid- to late gestation had offsprings with significantly lower weight at birth[72], and higher blood pressure in later life[70]. Babies born to mothers who were exposed to famine only during early gestation had normal birth weights. However, they had higher rates of obesity and cardiovascular and metabolic disease in adulthood[70, 72]. These results demonstrate that the programming of adulthood obesity by intrauterine adverse conditions is not always accompanied by lower birth weight[72]. Notably, in the

Dutch Hunger Winter study, exposure to famine was restricted to a certain time-period of pregnancy, whereas in other studies, exposures are often present from preconception until birth.

Within the DOHaD hypothesis, the placental 11beta-hydroxysteriod dehydrogenase type 2 (11βHSD2) enzyme plays a key role. Normally, in the placenta 11βHSD2 inactivates maternal cortisol[73-75], however 10-20% still reaches the fetus[76-78]. Cortisone can be considered an inactive metabolite of cortisol. Reverse metabolism from cortisone to cortisol occurs continuously[79]. Multiple maternal stress factors during pregnancy, like maternal smoking or alcohol use, psychological stress, famine/malnutrition, maternal disease, and the presence of pro-inflammatory cytokines are known to modulate the expression of 11βHSD2 (Figure 1).[78, 80, 81]. In addition, the capacity of 11βHSD2 may be exceeded by the administration of glucocorticoids[76, 77]. Subtle changes in 11βHSD2 activity during pregnancy, resulting in a relative deficiency of 11βHSD2, may lead to large glucocorticoid effects on the fetus.

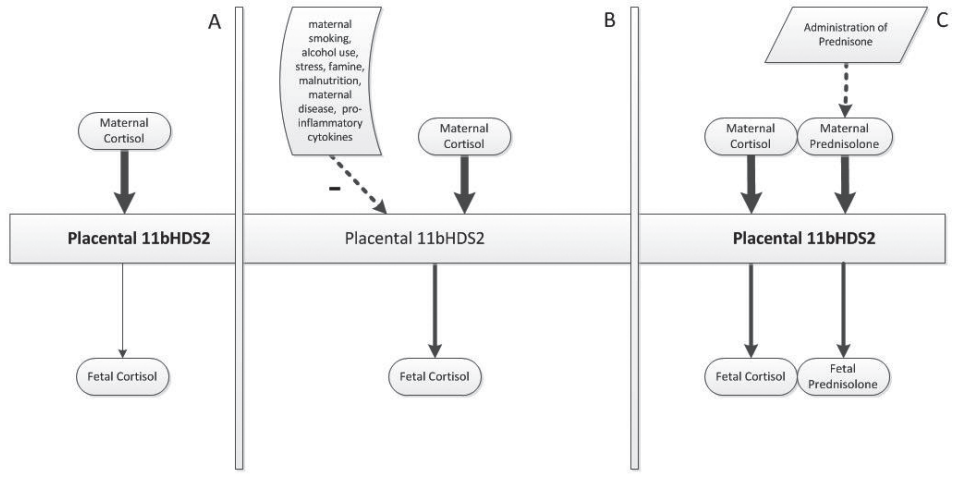


Figure 1. Factors that influence fetal cortisol concentrations. In (A) the normal physiological situation is shown. (B) illustrates the increased cortisol concentrations that reach the fetus because of decreased expression of the 11βHSD2 enzyme due to external factors . In (C) is shown that the administration of prednisone to the mother results in increased fetal glucocorticosteroids concentrations, namely cortisol and prednisolone when both exceed the capacity of 11βHSD2.

11βHSD2: 11beta-hydroxysteriod dehydrogenase type 2

1.4 CORTISOL AND PREGNANCY

1.4.1 Glucocorticoids and cortisol

Cortisol, a glucocorticoid hormone, is the final product of activation of the hypothalamic-pituitary-adrenal (HPA axis) and regulates among others the glucose and fat metabolism, the immune system, and the electrolyte balance[82-84]. Cortisol is also known as the stress hormone because it's released in higher doses under stressful conditions[85, 86]. Chronically elevated levels of this hormone are associated with an increased risk of cardiovascular and metabolic disease[87]. In the placenta the 11βHSD2 enzyme inactivates maternal cortisol by converting it to the inactive form cortisone. In normal situations, maternal cortisol rises during pregnancy along with a rise in placental 11βHSD2[88]. Towards the end of pregnancy the levels of 11βHSD2 drop, ensuring that the fetus is exposed to sufficient levels of cortisol, which are important for e.g. maturation of the fetal lungs[88].

Prednisone is a synthetic glucocorticoid pro-drug that is converted to the active form prednisolone in the liver, which supresses the HPA axis activity of the patient[89, 90]. The 11βHSD2 enzyme also inactivates prednisolone[73-75]. Factors known to influence the activity of this enzyme, as mentioned in paragraph 1.3 (e.g. maternal smoking, malnutrition, maternal disease and pro-inflammatory cytokines) and the administration of glucocorticoids, may lead to higher cortisol (and cortisone) concentrations in the offspring, which in turn may result in negative effects on the development of the fetal HPA axis[48, 89, 91, 92].

Exposure to higher cortisol concentrations in utero are associated with a lower birth weight and higher cortisol concentrations in the offsprings due to dysregulation of the fetal HPA axis[91, 93]. In addition, antenatal prednisone exposure in children born to female patients with RA is associated with higher daytime cortisol levels[81].

1.4.2 Long-term cortisol and cortisone analysis

Suitable matrices to test acute or short-term changes in cortisol include saliva, serum and urine. However, due to the circadian rhythm, pulsatile secretion, daily variation and reactivity to acute (transient) stress, none of these are useful for long-term cortisol analysis[85, 94]. Measuring cortisol and cortisone in hair is a relatively new, reliable and non-invasive method for long-term cortisol analysis[95-99]. Because hair grows approximately 1 cm per month, a hair sample of 1 cm is thought to reflect the mean exposure of free cortisol in 1 month[89, 94]. Additionally, sampling of hair that is taken from the posterior vertex is the most optimal, because the intra-individual variation is smallest at that site[95, 98]. Hair growth patterns vary across different regions of the scalp and the posterior vertex region shows the most uniform growth rates[100]. The mechanism of cortisol incorporation into hair is not fully understood. It is assumed that unbound cortisol passively diffuses from capillaries into the cells of the hair follicle and becomes deposited within the hair shaft[73, 95, 99]. Another possible mechanism is the incorporation of cortisol

from sweat or sebum secretions into the hair shaft[101]. There is a wide consensus that the first proximal 6 cm of hair can reliably reflect cortisol concentrations in the last 6 months. Exogenous environmental factors, such as frequent hair washing and cosmetic treatments decrease cortisol levels in the more distal segments of the hair. Theoretically, the older (distal) hair segments are more damaged due to environmental factors and cortisol could escape from these damaged hair segments especially during the washing procedures[87, 95, 102].

1.5 EPIGENETICS

Many diseases have a genetic background. The human genome consists of deoxyribonucleic acid (DNA), where genetic information is stored. Nucleotides, the building blocks of DNA, are composed of a nucleobase (cytosine (C), guanine (G), adenine (A), or thymine (T)), a sugar molecule and a phosphate group.

Genetic variations can be divided into DNA sequence variations, structural/chromosomal variations and epigenetic variations. In majority, common variations in DNA sequence are caused by single nucleobase substitutions (mutations), that can cause altered protein transcription (Figure 2). The term single nucleotide polymorphism (SNP) is used to describe mutations that occur relatively frequent in the population. Structural variations affect larger segments of DNA or whole chromosomes.

Epigenetic variations are stable, tissue-specific, environmentally influenced, sometimes reversible and to some extent heritable changes in a chromosome without alterations in the underlying DNA nucleotide sequence, that can result in altered gene expression[103]. These changes include DNA methylation, posttranslational histone modifications (e.g. acetylation, methylation, ubiquitination, phosphorylation) and non-coding microRNAs[103]. Epigenetic regulations are involved in determining gene functions and activities[104].

Although different autoimmune diseases have diverse epidemiology or symptoms, they usually have a common origin. Genetic predisposition is involved in the etiology and pathology of autoimmune disorders[105]. Twin studies showed that the contribution of genetics to RA is approximately 65%[106]. However, monozygotic twins, who share an identical genetic profile, have a low concordance rate of 12.3% in RA[107]. Discordance in the onset of autoimmune diseases in monozygotic twins suggests that factors other than genetics also have a predisposing influence[105, 107]. The environment plays a large role in determining an individual's phenotype through epigenetic mechanisms[107].

Epigenetics may be seen as a bridge in the gap between genetics and environment and can partially explain the discordance of some diseases in monozygotic twins[107]. Epigenetic modifications are believed to be the underlying mechanisms of biological fetal programming[69, 108-112] explaining the developmental origins of disease.

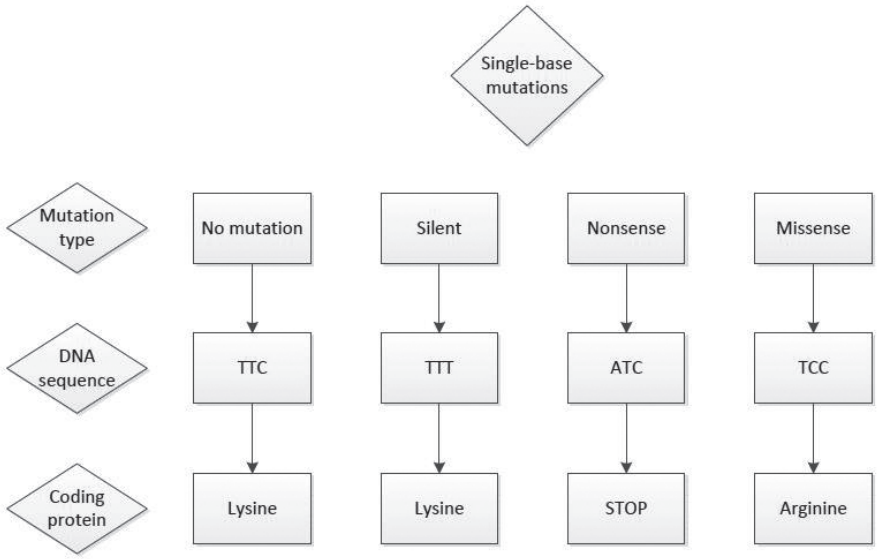


Figure 2. Examples of single nucleobase substitutions, resulting in silent, nonsense and missense mutations. Silent mutations are substitutions that result in coding for the same amino acid. Nonsense mutations are substitutions that result in coding for a stop codon and missense mutations are substitutions that result in coding for another amino acid.

1.5.1 DNA methylation

Among all the epigenetic mechanisms, DNA methylation is most extensively studied, but still not completely understood[113]. In DNA methylation the DNA sequence is not changed. Methylation mostly occurs at the 5' position on cytosine bases, where a methyl group is added to a cytosine converting it to a methyl-cytosine (Figure 3). DNA methyltransferase enzymes are responsible for the establishment and maintenance of methylation[105, 114]. This process usually occurs in regions of clustered Cytosine-phosphate-Guanine (CpG) dinucleotides, known as CpG islands (CGIs)[114, 115]. CGIs are mostly found proximal to promoters of housekeeping genes[115]. Changes in DNA methylation include hypermethylation and hypomethylation of CGIs[114, 115], and the effects these changes on the expression of genes depends on the location. Overall, hypermethylated regions block the accessibility of transcriptional factors and thereby inhibit gene transcription[105]. In contrast, regions with hypomethylation are accessible for transcription, resulting in enhanced transcription[116].

As mentioned before, during fetal development internal and external factors might have critical influences for long-term health. After fertilization, DNA methylation marks from the parents are erased by a process of almost full demethylation, with the exception of some

imprinted genes[117]. Subsequently, re-establishment of DNA methylation marks occurs with de novo methylation, mostly in the first weeks of pregnancy[117]. In this period, several factors like maternal disease[68] or malnutrition[69, 110], smoking[118], folate depletion[109] can influence DNA methylation of the offspring in utero, resulting in differential expression of genes and creating possible long-term adverse effects for the child[119].

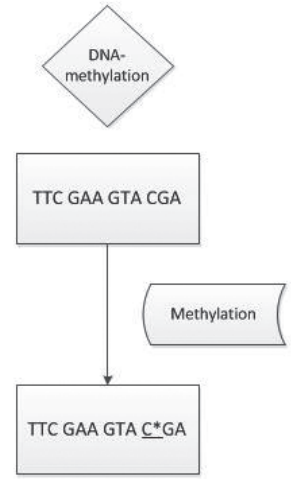


Figure 3. DNA methylation of a cytosine. DNA methylation does not alter the DNA nucleobase sequence. C illustrates an unmethylated and C* a methylated cytosine.

1.6 DESIGN OF THE STUDIES

1.6.1 PARA-study

The Pregnancy-induced Amelioration of Rheumatoid Arthritis (PARA) study is a large nationwide prospective cohort study conducted in the Netherlands[31]. The aims were to prospectively study the effects of pregnancy on RA and vice versa, including the pregnancy outcome. In the PARA-study female patients with RA who fulfilled the 1987 revised criteria of the American College of Rheumatology[120] and had a wish to conceive or were already pregnant were included from 2002 to 2008[31]. Patients were seen at preconception (if possible), 3 times during pregnancy and 3 times postpartum in a home-visit. During every visit data on mother (e.g. disease activity and medication use) and child (e.g. gestational age, birthweight) was collected[31]. For calculating RA disease activity the Disease Activity Score in 28 joints using C-Reactive Protein (DAS28-CRP(3)) was used[121].

1.6.2 FEPRA-study

A few years after participation in the PARA-study, mothers were asked to participate with their children in a follow-up study, the FEtal Programming in Rheumatoid Arthritis (FEPRA) study[122]. Growth charts and information on growth of the children were collected from birth and onwards. After reaching the age of 5, 108 children and their parents visited the Erasmus Medical Center (MC) Sophia Children's Hospital in Rotterdam. During this visit, anthropometric measurements (e.g. weight, height, blood pressure, waist and hip circumference and skin folds) and a Dual-Energy X-ray Absorptiometry (DXA)-scan were performed. In addition, saliva samples to measure cortisol and fasting blood samples to measure glucose and lipid levels, and to measure DNA methylation were collected[122].

1.6.3 HAIR-study

In 2014 the HAIR-study was conducted to study the long-term effects of antenatal prednisone exposure in children, aged 5-16 years, born to women with an autoimmune disease (primarily RA). Children with antenatal prednisone exposure were compared to children without antenatal exposure, all born to women with an autoimmune disorder. First, participants of the PARA-study were contacted for inclusion. Second, siblings of participants of the PARA-study were included. Finally, children born to women with an autoimmune disease from the outpatient clinic at the department of Rheumatology in the Erasmus MC were included. Participants were visited at home, where a tuft of hair from the children and data from children (weight, height, blood pressure, waist and hip circumference, skin folds) and their parents (weight, height, blood pressure) were collected.

1.7 AIMS AND OUTLINE OF THIS THESIS

In this thesis we have focused more in depth on the impact of RA during pregnancy on mother and child. Children born to women with RA have a less fortunate start compared to children born to women from the general population. They have a higher risk of lower birth weight with a rapid catch-up growth, both risk factors for future diseases. High RA disease activity and prednisone use have been shown to be associated with less favorable pregnancy outcomes.

Therefore, one of the main aims of the current study was to identify clinical factors at the beginning of pregnancy that could predict women who are more and women who are less likely to improve during pregnancy. Identifying those patients will help in clinical practice and in future studies to select patients in whom medication may be tapered, continued or intensified.

Another aim was to identify the possible long-term consequences of RA, RA disease activity and prednisone use on the offsprings, regarding DNA methylation and long-term cortisol and cortisone concentrations. Identifying these consequences will help to determine the impact of maternal disease on the later-life health of the offspring.

In addition, since infants with a low birth weight would benefit the most from breastmilk, studying lactation in this group of patients, and determining factors associated with discontinuation of breastfeeding is important. If we can identify influenceable reasons that prevent women with RA from breastfeeding, we might be able to increase the percentage and duration of lactation in female patients with RA.

Chapter 1 introduces the topics described in this thesis. In **Chapter 2** multiple aspects on RA and pregnancy, from fertilization to outcome, are reviewed. **Chapter 3** illustrates RA disease activity in two subsequent pregnancies to determine whether the course of the RA disease activity in a first pregnancy (during pregnancy and after delivery) is comparable with the disease course in a second pregnancy. The objective of **Chapter 4** was to determine a combination of clinical factors at the beginning of pregnancy, associated with low disease activity and remission of RA in the third trimester during pregnancy. Also to identify women who are more and less likely to improve during pregnancy. **Chapter 5** investigates whether children born to women with RA have a different DNA methylation profile compared to children born to women from the general population and whether these differences are associated with RA disease activity or medication use. **Chapter 6** focusses on the long-term consequences of antenatal prednisone exposure and RA disease activity to investigate whether these are associated with chronically elevated cortisol and cortisone levels in children born to women with RA. In addition, whether these are associated with a high-risk profile for metabolic and cardiovascular disease. **Chapter 7** demonstrates the differences in frequency and duration of breastfeeding of the offspring in female patients with RA versus the general population. Additionally, it demonstrates which clinical factors in the third trimester are associated with discontinuation of breastfeeding within 12 weeks. In **Chapter 8** we discuss the relevance and clinical implications of the results we've found. In **Chapter 9**, an English summary and in **Chapter 10** a Dutch summary of the dissertation is provided.

REFERENCES

1. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet 2010;376:1094-108.
2. Das S, Padhan P. An Overview of the Extraarticular Involvement in Rheumatoid Arthritis and its Management. J Pharmacol Pharmacother 2017;8:81-6.
3. Alamanos Y, Voulgari PV, Drosos AA. Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review. Semin Arthritis Rheum 2006;36:182-8.
4. Silman AJ, Newman J, MacGregor AJ. Cigarette smoking increases the risk of rheumatoid arthritis. Results from a nationwide study of disease-discordant twins. Arthritis Rheum 1996;39:732-5.
5. Angelotti F, Parma A, Cafaro G, Capecchi R, Alunno A, Puxeddu I. One year in review 2017: pathogenesis of rheumatoid arthritis. Clin Exp Rheumatol 2017;35:368-78.
6. Turesson C, O'Fallon WM, Crowson CS, Gabriel SE, Matteson EL. Occurrence of extraarticular disease manifestations is associated with excess mortality in a community based cohort of patients with rheumatoid arthritis. J Rheumatol 2002;29:62-7.
7. Turesson C, Jacobsson LT, Sturfelt G, Matteson EL, Mathsson L, Ronnelid J. Rheumatoid factor and antibodies to cyclic citrullinated peptides are associated with severe extra-articular manifestations in rheumatoid arthritis. Ann Rheum Dis 2007;66:59-64.
8. Hargreaves E. A survey of rheumatoid arthritis in West Cornwall; a report to the Empire Rheumatism council. . Ann Rheum Dis 1958;17:61-75.
9. Wallenius M, Skomsvoll JF, Irgens LM, Salvesen KA, Nordvag BY, Koldingsnes W, et al. Fertility in women with chronic inflammatory arthritides. Rheumatology (Oxford) 2011;50:1162-7.
10. Wallenius M, Skomsvoll JF, Irgens LM, Salvesen KA, Nordvag BY, Koldingsnes W, et al. Parity in patients with chronic inflammatory arthritides childless at time of diagnosis. Scand J Rheumatol 2012;41:202-7.
11. Skomsvoll JF, Ostensen M, Baste V, Irgens LM. Number of births, interpregnancy interval, and subsequent pregnancy rate after a diagnosis of inflammatory rheumatic disease in Norwegian women. J Rheumatol 2001;28:2310-4.
12. Brouwer J, Hazes JM, Laven JS, Dolhain RJ. Fertility in women with rheumatoid arthritis: influence of disease activity and medication. Ann Rheum Dis 2014;in press.
13. Del Junco DJ, Annegers JF, Coulam CB, Luthra HS. The relationship between rheumatoid arthritis and reproductive function. Br J Rheumatol 1989;28 Suppl 1:33; discussion 42-5.
14. Provost M, Eaton JL, Clowse ME. Fertility and infertility in rheumatoid arthritis. Curr Opin Rheumatol 2014;26:308-14.
15. Brouwer J, Laven JS, Hazes JM, Schipper I, Dolhain RJ. Levels of serum anti-Mullerian hormone, a marker for ovarian reserve, in women with rheumatoid arthritis. Arthritis Care Res (Hoboken) 2013;65:1534-8.
16. Brouwer J., Hazes J.M., Laven J.S., R.J. D. Overian reserve, as assessed by measuring serum Anti-Müllerian Hormone levels, declines more rapidly over time in Rheumatoid Arthritis patients compared to controls. Annals of the Rheumatic Diseases 2017;76:532.
17. Hench PS. The ameliorating effect of pregnancy on chronic atrophic (infectious rheumatoid) arthritis, fibrositis and intermittent hydrarthritis. Mayo Clin Proc 1938;13:161-7.
18. Lewis-Faning E. Report on an enquiry into the aetiological factors associated with rheumatoid arthritis. Ann Rheum Dis 1950;9 (Suppl).
19. Torrent PB, Marcet CA. Effect of pregnancy on the course of chronic progressive polyarthritis. Rev Esp Reum Enferm Osteoartic 1951;4:96-9.
20. Oka M. Effect of pregnancy on the onset and course of rheumatoid arthritis. Ann Rheum Dis 1953;12:227-9.
21. Betson JR, Jr., Dorn RV. Forty Cases of Arthritis and Pregnancy. J Int Coll Surg 1964;42:521-6.
22. Morris WI. Pregnancy in rheumatoid arthritis and systemic lupus erythematosus. Aust N Z J Obstet Gynaecol 1969;9:136-44.
23. Neely NT, Persellin RH. Activity of rheumatoid arthritis during pregnancy. Tex Med 1977;73:59-63.
24. Ostensen M, Aune B, Husby G. Effect of pregnancy and hormonal changes on the activity of rheumatoid arthritis. Scand J Rheumatol 1983;12:69-72.

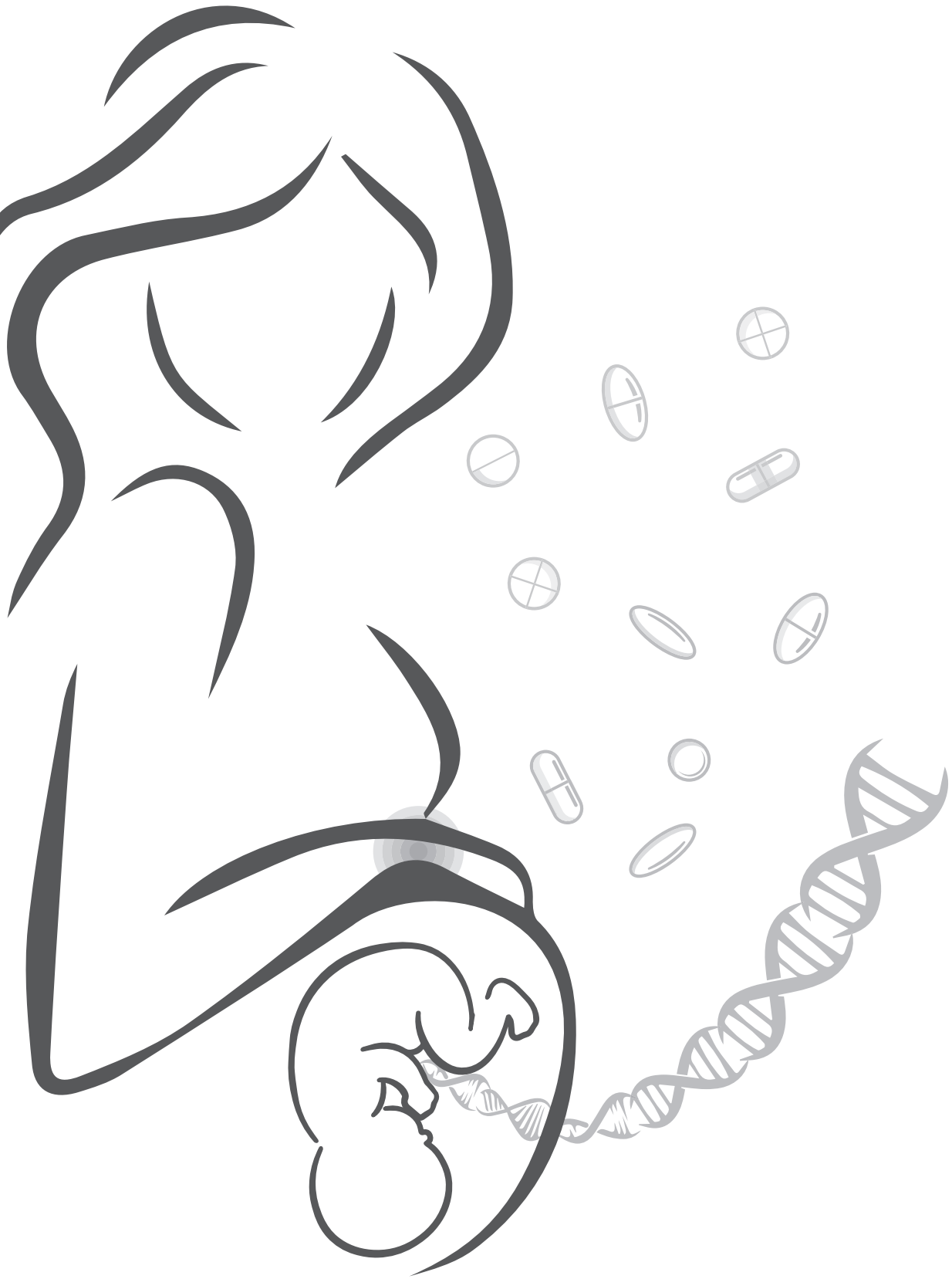
25. Klipple GL, Cecere FA. Rheumatoid arthritis and pregnancy. Rheum Dis Clin North Am 1989;15:213-39.
26. Ostensen M, Husby G. A prospective clinical study of the effect of pregnancy on rheumatoid arthritis and ankylosing spondylitis. Arthritis Rheum 1983;26:1155-9.
27. Smith WD, West HF. Pregnancy and rheumatoid arthritis. Acta Rheumatol Scand 1960;6:189-201.
28. Unger A, Kay A, Griffin AJ, Panayi GS. Disease activity and pregnancy associated alpha 2-glycoprotein in rheumatoid arthritis during pregnancy. Br Med J (Clin Res Ed) 1983;286:750-2.
29. Nelson JL, Hughes KA, Smith AG, Nisperos BB, Branchaud AM, Hansen JA. Maternal-fetal disparity in HLA class II alloantigens and the pregnancy-induced amelioration of rheumatoid arthritis. N Engl J Med 1993;329:466-71.
30. Barrett JH, Brennan P, Fiddler M, Silman AJ. Does rheumatoid arthritis remit during pregnancy and relapse postpartum? Results from a nationwide study in the United Kingdom performed prospectively from late pregnancy. Arthritis Rheum 1999;42:1219-27.
31. de Man YA, Dolhain RJ, van de Geijn FE, Willemsen SP, Hazes JM. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum 2008;59:1241-8.
32. Ince-Aşkan H, Dolhain RJ. Pregnancy and rheumatoid arthritis. Best Pract Res Clin Rheumatol 2015;29:580-96.
33. de Man YA, Bakker-Jonges LE, Goorbergh CM, Tillemans SP, Hooijkaas H, Hazes JM, et al. Women with rheumatoid arthritis negative for anti-cyclic citrullinated peptide and rheumatoid factor are more likely to improve during pregnancy, whereas in autoantibody-positive women autoantibody levels are not influenced by pregnancy. Ann Rheum Dis 2010;69:420-3.
34. Ostensen M, Villiger PM. The remission of rheumatoid arthritis during pregnancy. Semin Immunopathol 2007;29:185-91.
35. Amin S, Peterson EJ, Reed AM, Mueller DL. Pregnancy and rheumatoid arthritis: insights into the immunology of fetal tolerance and control of autoimmunity. Curr Rheumatol Rep 2011;13:449-55.
36. Ostensen M. Sex hormones and pregnancy in rheumatoid arthritis and systemic lupus erythematosus. Ann N Y Acad Sci 1999;876:131-43; discussion 44.
37. Kanik KS, Wilder RL. Hormonal alterations in rheumatoid arthritis, including the effects of pregnancy. Rheum Dis Clin North Am 2000;26:805-23.
38. Whitacre CC. Sex differences in autoimmune disease. Nat Immunol 2001;2:777-80.
39. Hazes JM, van Zeben D. Oral contraception and its possible protection against rheumatoid arthritis. Ann Rheum Dis 1991;50:72-4.
40. Fischer-Betz R, Specker C. Pregnancy in systemic lupus erythematosus and antiphospholipid syndrome. Best Pract Res Clin Rheumatol 2017;31:397-414.
41. Wolfberg AJ, Lee-Parritz A, Peller AJ, Lieberman ES. Association of rheumatologic disease with preeclampsia. Obstet Gynecol 2004;103:1190-3.
42. Norgaard M, Larsson H, Pedersen L, Granath F, Askling J, Kieler H, et al. Rheumatoid arthritis and birth outcomes: a Danish and Swedish nationwide prevalence study. J Intern Med 2010;268:329-37.
43. de Man YA, Hazes JM, van der Heide H, Willemsen SP, de Groot CJ, Steegers EA, et al. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: results of a national prospective study. Arthritis Rheum 2009;60:3196-206.
44. Bharti B, Lee SJ, Lindsay SP, Wingard DL, Jones KL, Lemus H, et al. Disease Severity and Pregnancy Outcomes in Women with Rheumatoid Arthritis: Results from the Organization of Teratology Information Specialists Autoimmune Diseases in Pregnancy Project. J Rheumatol 2015;42:1376-82.
45. Reed SD, Vollan TA, Svec MA. Pregnancy outcomes in women with rheumatoid arthritis in Washington State. Matern Child Health J 2006;10:361-6.
46. Brouwer J, Laven JS, Hazes JM, Dolhain RJ. Brief Report: Miscarriages in Female Rheumatoid Arthritis Patients: Associations With Serologic Findings, Disease Activity, and Antirheumatic Drug Treatment. Arthritis Rheumatol 2015;67:1738-43.
47. de Steenwinkel FD, Hokken-Koelega AC, de Ridder MA, Hazes JM, Dolhain RJ. Rheumatoid arthritis during pregnancy and postnatal catch-up growth in the offspring. Arthritis Rheumatol 2014;66:1705-11.
48. Ong KK, Dunger DB. Perinatal growth failure: the road to obesity, insulin resistance and cardiovascular disease in adults. Best Pract Res Clin Endocrinol Metab 2002;16:191-207.

49. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. JAMA 2009;301:2234-42.
50. Daly A, Pollard CM, Phillips M, Binns CW. Benefits, barriers and enablers of breastfeeding: factor analysis of population perceptions in Western Australia. PLoS One 2014;9:e88204.
51. Vieira Borba V, Sharif K, Shoenfeld Y. Breastfeeding and autoimmunity: Programing health from the beginning. Am J Reprod Immunol 2018;79.
52. Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, et al. Breastfeeding and maternal and infant health outcomes in developed countries. Evid Rep Technol Assess (Full Rep) 2007;in press:1-186.
53. Gharib S, Fletcher M, Tucker R, Vohr B, Lechner BE. Effect of Dedicated Lactation Support Services on Breastfeeding Outcomes in Extremely-Low-Birth-Weight Neonates. J Hum Lact 2017;in press:890334417741304.
54. Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. Pediatrics 2005;115:1367-77.
55. Owen CG, Whincup PH, Kaye SJ, Martin RM, Davey Smith G, Cook DG, et al. Does initial breastfeeding lead to lower blood cholesterol in adult life? A quantitative review of the evidence. Am J Clin Nutr 2008;88:305-14.
56. Barrett JH, Brennan P, Fiddler M, Silman A. Breast-feeding and postpartum relapse in women with rheumatoid and inflammatory arthritis. Arthritis Rheum 2000;43:1010-5.
57. Ostensen M, Khamashta M, Lockshin M, Parke A, Brucato A, Carp H, et al. Anti-inflammatory and immunosuppressive drugs and reproduction. Arthritis Res Ther 2006;8:209.
58. Weber-Schoendorfer C, Chambers C, Wacker E, Beghin D, Bernard N, Network of French Pharmacovigilance C, et al. Pregnancy outcome after methotrexate treatment for rheumatic disease prior to or during early pregnancy: a prospective multicenter cohort study. Arthritis Rheumatol 2014;66:1101-10.
59. Visser K, Katchamart W, Loza E, Martinez-Lopez JA, Salliot C, Trudeau J, et al. Multinational evidence-based recommendations for the use of methotrexate in rheumatic disorders with a focus on rheumatoid arthritis: integrating systematic literature research and expert opinion of a broad international panel of rheumatologists in the 3E Initiative. Ann Rheum Dis 2009;68:1086-93.
60. Gotestam Skorpen C, Hoeltzenbein M, Tincani A, Fischer-Betz R, Elefant E, Chambers C, et al. The EULAR points to consider for use of antirheumatic drugs before pregnancy, and during pregnancy and lactation. Ann Rheum Dis 2016;75:795-810.
61. Barouki R, Gluckman PD, Grandjean P, Hanson M, Heindel JJ. Developmental origins of non-communicable disease: implications for research and public health. Environ Health 2012;11:42.
62. Singhal A. Early Life Origins of Obesity and Related Complications. Indian J Pediatr 2017;in press.
63. Barker DJ. Fetal origins of cardiovascular disease. Ann Med 1999;31 Suppl 1:3-6.
64. de Boo HA, Harding JE. The developmental origins of adult disease (Barker) hypothesis. Aust N Z J Obstet Gynaecol 2006;46:4-14.
65. Painter RC, de Rooij SR, Bossuyt PM, Simmers TA, Osmond C, Barker DJ, et al. Early onset of coronary artery disease after prenatal exposure to the Dutch famine. Am J Clin Nutr 2006;84:322-7; quiz 466-7.
66. Stein AD, Kahn HS, Rundle A, Zybert PA, van der Pal-de Bruin K, Lumey LH. Anthropometric measures in middle age after exposure to famine during gestation: evidence from the Dutch famine. Am J Clin Nutr 2007;85:869-76.
67. Turan N, Ghalwash MF, Katari S, Coutifaris C, Obradovic Z, Sapienza C. DNA methylation differences at growth related genes correlate with birth weight: a molecular signature linked to developmental origins of adult disease? BMC Med Genomics 2012;5:10.
68. Hogg K, Price EM, Hanna CW, Robinson WP. Prenatal and perinatal environmental influences on the human fetal and placental epigenome. Clin Pharmacol Ther 2012;92:716-26.
69. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 2008;105:17046-9.
70. Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. Early Hum Dev 2006;82:485-91.
71. Tobi EW, Slieker RC, Luijk R, Dekkers KF, Stein AD, Xu KM, et al. DNA methylation as a mediator of the association between prenatal adversity and risk factors for metabolic disease in adulthood. Sci Adv 2018;4:eaao4364.
72. Schulz LC. The Dutch Hunger Winter and the developmental origins of health and disease. Proceedings of the National Academy of Sciences of the United States of America 2010;107:16757-8.

73. D'Anna-Hernandez KL, Ross RG, Natvig CL, Laudenslager ML. Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol. Physiol Behav 2011;104:348-53.
74. Benediktsson R, Calder AA, Edwards CR, Seckl JR. Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. Clin Endocrinol (Oxf) 1997;46:161-6.
75. Burton PJ, Waddell BJ. Dual function of 11beta-hydroxysteroid dehydrogenase in placenta: modulating placental glucocorticoid passage and local steroid action. Biol Reprod 1999;60:234-40.
76. de Steenwinkel FD, Hokken-Koelega AC, Hazes JM, Dolhain RJ. The influence of foetal prednisone exposure on the cortisol levels in the offspring. Clin Endocrinol (Oxf) 2013;in press.
77. Edwards CR, Benediktsson R, Lindsay RS, Seckl JR. Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? Lancet 1993;341:355-7.
78. Kossintseva I, Wong S, Johnstone E, Guilbert L, Olson DM, Mitchell BF. Proinflammatory cytokines inhibit human placental 11beta-hydroxysteroid dehydrogenase type 2 activity through Ca2+ and cAMP pathways. Am J Physiol Endocrinol Metab 2006;290:E282-8.
79. Rippe RC, Noppe G, Windhorst DA, Tiemeier H, van Rossum EF, Jaddoe VW, et al. Splitting hair for cortisol? Associations of socio-economic status, ethnicity, hair color, gender and other child characteristics with hair cortisol and cortisone. Psychoneuroendocrinology 2016;66:56-64.
80. Anwar MA, Saleh AI, Al Olabi R, Al Shehabi TS, Eid AH. Glucocorticoid-induced fetal origins of adult hypertension: Association with epigenetic events. Vascul Pharmacol 2016;82:41-50.
81. de Steenwinkel FD, Hokken-Koelega AC, Hazes JM, Dolhain RJ. The influence of foetal prednisone exposure on the cortisol levels in the offspring. Clin Endocrinol (Oxf) 2014;80:804-10.
82. Ferrari P. Cortisol and the renal handling of electrolytes: role in glucocorticoid-induced hypertension and bone disease. Best Pract Res Clin Endocrinol Metab 2003;17:575-89.
83. Khani S, Tayek JA. Cortisol increases gluconeogenesis in humans: its role in the metabolic syndrome. Clin Sci (Lond) 2001;101:739-47.
84. Jefferies WM. Cortisol and immunity. Med Hypotheses 1991;34:198-208.
85. Staufenbiel SM, Penninx BW, Spijker AT, Elzinga BM, van Rossum EF. Hair cortisol, stress exposure, and mental health in humans: a systematic review. Psychoneuroendocrinology 2013;38:1220-35.
86. Groeneveld MG, Vermeer HJ, Linting M, Noppe G, van Rossum EF, van IMH. Children's hair cortisol as a biomarker of stress at school entry. Stress 2013;16:711-5.
87. Manenschijn L, Koper JW, Lamberts SW, van Rossum EF. Evaluation of a method to measure long term cortisol levels. Steroids 2011;76:1032-6.
88. Davis EP, Sandman CA. The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. Child Dev 2010;81:131-48.
89. Karlen J, Frostell A, Theodorsson E, Faresjo T, Ludvigsson J. Maternal influence on child HPA axis: a prospective study of cortisol levels in hair. Pediatrics 2013;132:e1333-40.
90. Madsbad S, Bjerregaard B, Henriksen JH, Juhl E, Kehlet H. Impaired conversion of prednisone to prednisolone in patients with liver cirrhosis. Gut 1980;21:52-6.
91. Gelman PL, Flores-Ramos M, Lopez-Martinez M, Fuentes CC, Grajeda JP. Hypothalamic-pituitary-adrenal axis function during perinatal depression. Neurosci Bull 2015;31:338-50.
92. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 1993;36:62-7.
93. Green BB, Armstrong DA, Lesseur C, Paquette AG, Guerin DJ, Kwan LE, et al. The Role of Placental 11-Beta Hydroxysteroid Dehydrogenase Type 1 and Type 2 Methylation on Gene Expression and Infant Birth Weight. Biol Reprod 2015;92:149.
94. Veldhorst MA, Noppe G, Jongejan MH, Kok CB, Mekic S, Koper JW, et al. Increased scalp hair cortisol concentrations in obese children. J Clin Endocrinol Metab 2014;99:285-90.
95. Russell E, Koren G, Rieder M, Van Uum S. Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. Psychoneuroendocrinology 2012;37:589-601.
96. Gow R, Thomson S, Rieder M, Van Uum S, Koren G. An assessment of cortisol analysis in hair and its clinical applications. Forensic Sci Int 2010;196:32-7.

97. Kalra S, Einarson A, Karaskov T, Van Uum S, Koren G. The relationship between stress and hair cortisol in healthy pregnant women. Clin Invest Med 2007;30:E103-7.
98. Sauve B, Koren G, Walsh G, Tokmakejian S, Van Uum SH. Measurement of cortisol in human hair as a biomarker of systemic exposure. Clin Invest Med 2007;30:E183-91.
99. Xie Q, Gao W, Li J, Qiao T, Jin J, Deng H, et al. Correlation of cortisol in 1-cm hair segment with salivary cortisol in human: hair cortisol as an endogenous biomarker. Clin Chem Lab Med 2011;49:2013-9.
100. Pragst F, Balikova MA. State of the art in hair analysis for detection of drug and alcohol abuse. Clin Chim Acta 2006;370:17-49.
101. Stalder T, Kirschbaum C. Analysis of cortisol in hair--state of the art and future directions. Brain Behav Immun 2012;26:1019-29.
102. Dettenborn L, Tietze A, Kirschbaum C, Stalder T. The assessment of cortisol in human hair: associations with sociodemographic variables and potential confounders. Stress 2012;15:578-88.
103. Araki Y, Mimura T. The Mechanisms Underlying Chronic Inflammation in Rheumatoid Arthritis from the Perspective of the Epigenetic Landscape. J Immunol Res 2016;2016:6290682.
104. Ospelt C, Gay S, Klein K. Epigenetics in the pathogenesis of RA. Semin Immunopathol 2017;39:409-19.
105. Zhang Z, Zhang R. Epigenetics in autoimmune diseases: Pathogenesis and prospects for therapy. Autoimmun Rev 2015;14:854-63.
106. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 2000;43:30-7.
107. Xiang Z, Yang Y, Chang C, Lu Q. The epigenetic mechanism for discordance of autoimmunity in monozygotic twins. J Autoimmun 2017;83:43-50.
108. Foran E, Garrity-Park MM, Mureau C, Newell J, Smyrk TC, Limburg PJ, et al. Upregulation of DNA methyltransferase-mediated gene silencing, anchorage-independent growth, and migration of colon cancer cells by interleukin-6. Mol Cancer Res 2010;8:471-81.
109. Glier MB, Green TJ, Devlin AM. Methyl nutrients, DNA methylation, and cardiovascular disease. Mol Nutr Food Res 2014;58:172-82.
110. Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, et al. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Hum Mol Genet 2009;18:4046-53.
111. Crudo A, Petropoulos S, Moisiadis VG, Iqbal M, Kostaki A, Machnes Z, et al. Prenatal synthetic glucocorticoid treatment changes DNA methylation states in male organ systems: multigenerational effects. Endocrinology 2012;153:3269-83.
112. Park JH, Stoffers DA, Nicholls RD, Simmons RA. Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. J Clin Invest 2008;118:2316-24.
113. Oppermann U. Why is epigenetics important in understanding the pathogenesis of inflammatory musculoskeletal diseases? Arthritis Res Ther 2013;15:209.
114. Jin B, Li Y, Robertson KD. DNA methylation: superior or subordinate in the epigenetic hierarchy? Genes Cancer 2011;2:607-17.
115. Day K, Waite LL, Thalacker-Mercer A, West A, Bamman MM, Brooks JD, et al. Differential DNA methylation with age displays both common and dynamic features across human tissues that are influenced by CpG landscape. Genome Biol 2013;14:R102.
116. Ruemmele FM, Garnier-Lengline H. Why are genetics important for nutrition? Lessons from epigenetic research. Ann Nutr Metab 2012;60 Suppl 3:38-43.
117. Seisenberger S, Peat JR, Hore TA, Santos F, Dean W, Reik W. Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. Philos Trans R Soc Lond B Biol Sci 2013;368:20110330.
118. Joubert BR, Haberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, et al. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. Environ Health Perspect 2012;120:1425-31.
119. Kwon EJ, Kim YJ. What is fetal programming?: a lifetime health is under the control of in utero health. Obstet Gynecol Sci 2017;60:506-19.
120. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.

121. de Man YA, Hazes JM, van de Geijn FE, Krommenhoek C, Dolhain RJ. Measuring disease activity and functionality during pregnancy in patients with rheumatoid arthritis. Arthritis Rheum 2007;57:716-22.
122. de Steenwinkel FDO, Dolhain R, Hazes JMW, Hokken-Koelega ACS. Does prednisone use or disease activity in pregnant women with rheumatoid arthritis influence the body composition of their offspring? Reprod Toxicol 2017;71:118-23.



Pregnancy and rheumatoid arthritis

Review

Hilal Ince-Aşkan
Radboud J.E.M. Dolhain

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ABSTRACT

Fertility is impaired in female patients with rheumatoid arthritis (RA), which is related to disease activity and the use of certain medication. During pregnancy, disease activity usually improves, but less than previously thought. Especially in women with high disease activity, the pregnancy outcome is also impaired. All of this underscores the importance of strict control of disease activity in patients with RA who wish to conceive.

Management of RA disease activity during pregnancy might be a challenge as the treatment options are limited. Evidence is accumulating that tumor necrosis factor (TNF) blockers can be safely used during pregnancy, particularly during the first trimester and the beginning of the second trimester.

Far less is known about the problems faced by male RA patients who wish to conceive, in terms of not only fertility and pregnancy outcome, but also the safety of medication.

In this paper, the fertility issues in patients with RA, the pregnancy-associated improvement of RA, the pregnancy outcomes, including the long-term effects on the offspring, and treatment options, including those during lactation and for male patients wishing to conceive, will be reviewed.

CASE REPORT

Mrs. A, a 41-year-old patient with rheumatoid arthritis (RA), visited the specialized outpatient clinic of the Department of Rheumatology for consultation on her wish to conceive. Her medical history revealed diabetes mellitus type 1 since the age of 25. At the age of 33, she was diagnosed with a rheumatoid factor (RF)- and anti-citrullinated protein antibody (ACPA)-positive, erosive RA. She and her partner had been trying to conceive for more than 7 years, but this had not resulted in a live birth. The fertility work-up did not reveal any cause for the couple's subfertility. In their attempts to conceive, the couple had undergone intrauterine insemination (IUI) 6 times and an in vitro fertilization (IVF) procedure twice. In both IVF procedures, the embryos could be transferred into the uterine cavity. During her attempts to conceive, the patient was treated with sulfasalazine and etanercept, but she reported active RA during this period. At the age of 40, she spontaneously became pregnant when being treated with methotrexate (MTX) and etanercept. Unfortunately, this resulted in a miscarriage at week 7 of gestation.

At the first consultation, the patient still showed active RA (Disease Activity in 28 joints (DAS28) 3.5; 5 swollen joints) despite treatment with etanercept and MTX. MTX and etanercept were stopped, and the patient was started on 400 mg of infliximab (5 mg/ kg bodyweight) every 6 weeks, 1000 mg of sulfasalazine twice daily, and 400 mg of hydroxychloroquine once daily. Four months thereafter, the RA was in clinical remission (DAS28 2.6; 1 swollen joint); after careful consideration and gynecological consultation, IUI was restarted, as the couple decided not to undergo IVF anymore. The second IUI attempt resulted in a successful pregnancy. At week 20 of gestation, infliximab was stopped and 200 mg of certolizumab every second week was started. The patient gave birth to a healthy baby girl at week 38.0 of gestation. Both infliximab and certolizumab could not be detected in the cord blood. Throughout pregnancy, the RA remained in remission and the diabetes was well controlled. Ten weeks after delivery, the patient experienced a flare of her arthritis for which MTX was added to the combination therapy of certolizumab, sulfasalazine, and hydroxychloroquine. At the following visit, 3 months later, the RA was again in clinical remission.

FERTILITY IN PATIENTS WITH RA

Conceiving a child is a major life event, and most adults try to have a child during their reproductive life span. However, the literature suggests that achieving this goal might be more difficult for women with RA.

Several studies indicate that family size is diminished in women with RA[1-6]. Some studies[2, 6], but not all[5], indicate that this smaller family size is already present before the actual diagnosis of RA. Apart from a smaller family size in women with RA, such patients experience more difficulties in conceiving, as indicated by a longer time to pregnancy (TTP). In a study performed in the Danish National Birth Cohort, it was shown that 25% of patients with RA took more than a year to conceive compared with 15.6% of controls[7]. As only women who gave birth were included in this birth cohort, the actual data might be more concerning, as data on women who do not conceive at all were not incorporated. Clowse et al.[8] reported that 36% of patients with RA had difficulties in conceiving at least once during their reproductive life span. In the Pregnancy-induced Amelioration of Rheumatoid Arthritis (PARA) study, a prospective Dutch cohort study on RA and pregnancy[9], it was shown that 42% of patients with RA did not conceive within 1 year or at all during the follow-up period of that study. For comparison, in the general population, the median prevalence of subfertility defined as TTP of > 12 months is 9%, with a range of 3.5-24.2 % depending on the geographic area[10, 11].

Several factors might be associated with the reported smaller family size and the longer TTP in female patients with RA. As impaired fertility might already be present before the diagnosis of RA[2, 6] and as earlier menopause has been reported in patients with RA[2, 6], it has been postulated that these patients have a smaller ovarian reserve, which is already present before the onset of the disease and could account both for the impaired fertility and for the earlier menopause[12]. To test this hypothesis, the levels of serum anti-Müllerian hormone (AMH) (the most reliable biomarker for ovarian reserve) were tested in 72 premenopausal women (age range 18-42 years) with early RA. No differences were observed between patients with RA and healthy controls, making it unlikely that the observed impaired fertility, already present at time of diagnosis, is related to a diminished ovarian reserve[13]. Personal choices, due to RA related concerns, have been shown to be at least partially responsible for the smaller family size[8], but it cannot account for the observed impaired fertility. Inflammation, as reflected by disease activity, has been shown to be associated with TTP. In women with active disease (DAS28 > 5.1) TTP exceeded 1 year in 67% of women, whereas this was only 30% in women in remission (DAS28 < 2.6)[9]. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) has been associated with increased TTP[9], most likely through inhibition of the production of prostaglandins. Prostaglandins are involved in ovulation and implantation[12]. In addition, prednisone in a dose exceeding 7.5 mg daily has been associated with prolonged TTP[9]. The effect of prednisone might be related to a transient suppression of the hypothalamic-pituitary-ovarian axis by glucocorticoids or by a direct effect on ovarian function or on the endometrium[14, 15]. Finally, mainly based on studies in oncology and in animal models, it has been suggested that prior treatment with MTX is associated with impaired fertility[12]. However, in a prospective cohort study on RA and fertility, prior MTX

treatment did not affect TTP[9]. Furthermore, short-term MTX treatment had no impact on the ovarian reserve in patients patients with RA[13]. Finally, is has been hypothesized that the impaired fertility in patients with RA is a result of a lower intercourse frequency[12]. Although a high prevalence of sexual dysfunction has been described in mainly postmenopausal RA patients[12], it is unclear whether any conclusions can be drawn from these studies on the intercourse frequency of young patients with RA who actively wish to conceive.

In conclusion, the doctor must be aware of the impaired fertility and consider various contributing factors, when consulting an RA patient who wishes to conceive.

RA DISEASE COURSE DURING AND AFTER PREGNANCY

Determination of the RA disease activity during pregnancy

As disease activity is associated with TTP as well as pregnancy outcome in patients with RA, accurately determining RA disease activity in pregnancy is important. However, this is difficult as several measures of determining disease activity or its components are influenced by pregnancy. For example, the erythrocyte sedimentation rate (ESR) is elevated in pregnant women due to increased circulating fibrinogen, plasma expansion and decreased hemoglobin concentration[16]. The ESR rises during pregnancy from 10 mm/hour in the first trimester to 33 mm/hour in the third trimester, and it declines postpartum to <20mm/hour[17]. Therefore, variants of the disease activity score (DAS) that include the ESR are not preferred as scoring methods in pregnancy[17, 18]. Pregnancy might also influence the visual analogue scale (VAS) of global health, which is incorporated in the DAS[19]. These are some of the reasons for investigating validated alternative DAS scoring methods. Given that C-reactive protein (CRP) is only slightly influenced by pregnancy, RA disease activity during gestation can be most reliably determined with a DAS incorporating a swollen and tender joint count and a CRP, but without considering of the VAS scores[17, 20]. The Clinical Disease Activity Index (CDAI) and Simplified Disease Activity Index (SDAI) have not been validated for use in pregnancy and have not been used in pregnancy studies.

RA disease course during pregnancy

Pregnancy is the most studied physiological condition in which RA remits spontaneously[18, 21]. This phenomenon was first described by Hench in 1938[22]. In this retrospective study, improvement was observed in 90% of the 34 included pregnancies. Following this, multiple retrospective studies between 1950 and 1989 confirmed his initial observation with improvement rates in pregnant RA patients ranging from 54 to 95%[1, 23-30], (Table 1). Besides retrospective studies, a few small prospective studies[18, 31, 32] within that period of time

reported improvement in 71-86% of the patients. In 1993, Nelson et al.[33] showed remission in 39% and improvement in 21% of the 18 prospectively and 39 retrospectively followed pregnancies.

After this period, multiple larger prospective studies were conducted. Barrett et al.[21] prospectively studied 140 pregnant patients with RA in the UK to ascertain the influence of pregnancy on RA disease activity. Patients were followed up from the third trimester of their pregnancy until 6 months postpartum. In this study, the disease activity was assessed by clinical examination of inflamed joints and a VAS and Health Assessment Questionnaire (HAQ) scoring system. Data were collected at the third trimester of pregnancy and 1 and 6 months postpartum. The main disadvantage of this study is that the evaluation started at the third trimester, and therefore disease activity in the first and second trimester was retrospectively assessed by self-report. In this study, in total 65% of the patients retrospectively reported a decrease in pain and swelling during pregnancy. However, only 16% reached complete remission in the third trimester, defined as no swollen joints and receiving no antirheumatic therapy[21]. From 2002 until 2008, de Man et al.[34] conducted the PARA-study, a nationwide prospective cohort study within the Netherlands, to gain insight into the influence of pregnancy on the disease activity and the impact of RA and medication use on the pregnancy outcome. In this study, disease activity was objectively assessed with the validated DAS28-CRP(3) scoring system. The patients were followed up from preconception (if possible) up to 26 weeks postpartum. The results of the PARA-study showed that 48% of the 52 patients with an initial DAS28-CRP(3) ≥ 3.2 improved during pregnancy, based on the European League Against Rheumatism (EULAR) response criteria (good and moderate response combined). Improvement occurred although certain medications such as the contraindicated DMARDs were discontinued in all women wishing to conceive. When all patients, including those who were already in remission at the time of conception, were analyzed together, the mean DAS28 decreased from the preconception visit to third trimester by 0.4 (from 3.8 to 3.4) with a more pronounced decrease in patients with a moderate to high disease activity in the first trimester. Nevertheless, the percentage of patients with moderate to high disease activity (DAS28 ≥ 3.2) in the third trimester was approximately 50%. Furthermore, in the third trimester, in total 27% of the patients were in remission compared with 11% before conception, according to a DAS28 < 2.6[34].

The differences in improvement rates between earlier and more recent studies can be explained by differences in study design (retrospective vs. prospective studies) and in patients selection; in some earlier studies, only patients with active disease were included. Also various definitions of improvement and remission may contribute to differences in study outcome. In addition, increased numbers of patients with RA enter their pregnancy with low disease activity as treatment regiments have been intensified over the past decades.

Table 1. Studies on the effect of pregnancy in women with rheumatoid arthritis

Study	Study type	Number of patients (pregnancies)	Improvement during pregnancy, %	Deterioration postpartum, %
Hench[22]	Retrospective	20 (34)	90	90
Lewis-Faning[23]	Retrospective	22	95	81
Torrent[24]	Retrospective	15	80	-
Oka[25]	Retrospective	93 (114)	75	84
Hargreaves[1]	Retrospective	10 (11)	91	91
Smith[31]	Prospective	12	75	-
Betson[26]	Retrospective	24	54	-
Morris[27]	Retrospective	17	82	-
Neely[28]	Retrospective	20	63	-
Ostensen[29]	Retrospective	31 (49)	75	62
Ostensen[18]	Prospective	10	90	100
Unger[32]	Prospective	14	71	-
Klipple[30]	Retrospective	93 (114)	77	>90
Nelson[33]	Partial retrospective and prospective	41 (57, retrospective =18, prospective=39)	60 (remission 39%)	-
Barrett[21]	Prospective	140	65 (remission 16%)	62-77
De Man[34]	Prospective	84 total, 52 with initial DAS28-CRP(3) ≥3.2	48 (remission 27%)	39
De Man[35]	Prospective	118	39 and 75 (depending on the presence of autoantibodies)	34 and 42 (depending on the presence of autoantibodies)

RA disease course post-partum

After delivery, there is an increased risk of a flare in disease activity. Postpartum exacerbations were also described by Hench[22] in 90% of the 34 pregnancies. Other retrospective studies report percentages of patients with a flare between 62% and 90%[25, 29, 30] (Table 1). In the prospective study from Barrett et al.[21], 66% of the women reported an increase in joint swelling and 77% in pain at 6 months postpartum. Furthermore, in this study, 62% of the women had more affected joints postpartum than in the third trimester. The median number of affected joints increased from 8 during pregnancy to 10 at 6 months postpartum[21].

De Man et al.[34] reported that 39% of patients experienced a flare after delivery, despite restarting medication. Interestingly, also after miscarriage, one-third of patients experience a flare of their disease activity. In a recent study on a sample of 21 patient with RA, Brouwer et al.[36] showed that in 33% the disease flares between the preconception period and 3 months after a miscarriage.

In conclusion, it must be noted that RA improves less and in fewer patients during pregnancy than is generally perceived. Therefore, when patients with RA conceive successfully, medication must be continued and only tapered with caution. In addition, after delivery and even during lactation, continuation of appropriate medication might be necessary to prevent a flare of RA disease activity.

OUTCOMES

Pregnancy Outcomes

In large cohort studies, it has been found that pregnancy outcome in patients with RA is, in general, less favorable than in the normal healthy population[37-40], although not to the same extend as that reported for systemic lupus erythematosus (SLE) for example. Differences from the general population are often small; therefore, it is unclear whether the found differences have clinical implications for the individual patient. Notably, none of the studies on pregnancy outcome in female RA patients found increased risks of major congenital malformations or perinatal death[41, 42].

An overview of studies on RA and pregnancy outcome is shown in Table 2.

Miscarriage

Brouwer et al.[36] recently reported that the risk of a miscarriage in women with RA (17%) is comparable to that in the general population (11-22%). However, they do note that the miscarriage rate in patients with RA might be an underestimation. They explain that, among other reasons, the patients in their cohorts had planned pregnancies and therefore did not use any medication, for example, MTX, which is associated with an increased risk of miscarriages, and that there were fewer smokers and more patients with higher education levels in this cohort than in the general population[36].

Preeclampsia

Preeclampsia is a relatively uncommon pregnancy complication. Wolfberg et al.[41] showed an increased risk of preeclampsia in 114 women with rheumatic disease (e.g. RA and SLE) compared with unaffected women (8.8% vs. 2.3%). In a large combined Swedish and Danish prevalence study from 1994 to 2006 on 1199 women with RA and a first-time singleton birth registered in population-based healthcare databases, Norgaard et al.[38] showed that the risk of preeclampsia increased slightly from 3.4% in unaffected women to 5.0% in women with RA. Some studies[43] did not report an increase in preeclampsia during pregnancy in patients with RA, but this is most likely the result of a lack of power.

Mode of delivery

In a cohort of 243 women from the Washington State birth records, Reed et al.[42] found that women with RA had increased risks of a primary cesarean section (34% versus 19.5% in the control group). The main reasons for cesarean section in the RA population were cephalopelvic disproportion, dysfunctional labor, and fetal distress in 32%, 31%, and 18% respectively. These percentages were not significantly different from women without RA[42]. In the previously mentioned study by Norgaard et al.[38], patients with RA had more cesarean deliveries (26.0% versus 16.5% in unaffected controls). However they did not report the indications for cesarean sections. In the study by de Man et al.[43], cesarean sections were performed more often in the group with DAS28-CRP(3) ≥ 3.2 (22% versus 10% in the group with DAS28-CRP(3) < 3.2) and an increased number of patients with RA needed an instrumental vaginal delivery (17% vs. 10% in the reference group), but the latter was not associated with disease activity. Cesarean sections were indicated in the majority of the patients due to breech delivery and failure of labor progression[43]. Data on cesarean delivery should be interpreted with caution, as indication to perform a cesarean section may vary for different countries.

Preterm delivery

The definition of preterm delivery in these studies is a birth before 37 weeks of gestation. In the previously mentioned study by Wolfberg et al.[41] and Norgaard et al.[38] the risk of preterm delivery was increased in women with RA compared with healthy controls (15.2% vs. 7.8% and 9.2% vs. 6.2%, respectively). Other studies[37, 42] report comparable risk percentages. In a recent prospective cohort study on 440 pregnant women with RA within the Organization of Teratology Information Specialists Autoimmune Diseases in Pregnancy Project, Bharti et al.[40] showed that high disease severity (as defined by a HAQ Disability Index (HAQ-DI) > 0.5) is associated with preterm delivery (20% vs. 12% in mild disease). In the PARA-study by de Man et al.[43], the use of prednisone was associated with shorter gestational age (< 37 weeks) of the offspring at delivery.

Small-for-gestational-age infants

As shown in table 2 multiple studies[37-41] report an increased risk of small-for-gestational-age (SGA) infants ranging from +/- 3% in unaffected women to +/- 10% in women with RA. Some studies defined SGA as a birth weight more than 2 standard deviations below the mean for infants of similar gestational age[38, 43], and others as a birth weight lower than the 10th percentile adjusted for the gestational age[37, 39, 40]. De Man et al.[43] demonstrated that an increase in RA disease activity during pregnancy is independently associated with lower birth weight in the offspring, although still within the normal range. In this study, the incidence of SGA infants was 3.3%[43].

Table 2. Overview of studies on pregnancy outcome in female RA patients

Study	Study type	Number of patients	Findings on pregnancy outcome
Nelson[44]	Case-control	144 female RA patients versus 605 unaffected female controls	No increased risk: - spontaneous abortions - stillbirths
Skomsvoll[37]	Retrospective (birth registry)	4716 female patients with inflammatory arthritis (RA, juvenile RA, ankylosing spondylitis) versus 1,645,029 unaffected female controls	Increased risk: - preterm delivery (7.1% vs. 5.6%) - SGA infants (9.9% vs. 8.7%)
Wolfberg[41]	Retrospective (birth registry)	114 female RA patients with rheumatic disease (e.g. RA, systemic lupus erythematosus) versus 18,534 unaffected female controls	Increased risk: - preterm delivery (15.2% vs. 7.8%) - SGA offsprings (8.0% vs. 3.1%) - preeclampsia (15.2% vs. 7.8%)
Reed[42]	Retrospective (birth registry)	243 female RA patients versus 2559 unaffected female controls	Increased risk: - preterm delivery (13.6% vs. 7.2%) - cesarean section (34% vs. 19.5%) No increased risk: - preeclampsia
Norgaard[38]	Retrospective (birth registry)	1199 female RA patients versus 870,380 unaffected female controls	Increased risk: - preterm delivery (9.2% vs. 6.2%) - SGA infants (5.9% vs. 3.8%) - cesarean section (26.0% vs. 16.5%) - preeclampsia (5.0% vs. 3.4%)
Lin[39]	Retrospective (birth registry)	1912 female RA patients versus 9560 matched controls	Increased risk: - SGA infants (17.3% vs. 14.9%) - cesarean section (42% vs. 37.7%) - preeclampsia (2.7% vs. 1.2%)
de Man[43]	Prospective	152 female RA patients versus 175,498 unaffected female controls (from the Netherlands Perinatal Registry (PRN))	Increased risk: - instrumental vaginal delivery (17% vs. 10% in the PRN) - cesarean sections group with DAS28-CRP(3) ≥3.2 (22% vs. 10% in group with DAS28-CRP(3) <3.2) - SGA infants 3.3% No increased risk: - gestational hypertension - preeclampsia

Table 2. Continued

Study	Study type	Number of patients	Findings on pregnancy outcome
Langen[45]	Retrospective	46 female RA patients	Increased risk: - preterm delivery (<37 weeks) 28%
Wallenius[46]	Retrospective (birth registry)	1496 female RA patients versus general Norwegian population	Increased risk: - vaginal bleeding (adjusted odds ratio (aOR) 1.8) - elective cesarean section (aOR 2.0) - preterm delivery (aOR 1.5)
Rom[47]	Retrospective (birth registry)	2101 female RA patients versus 1,915,622 unaffected female controls	Increased risk: - preterm delivery (aOR 1.48)
Bharti[40]	Prospective	440 female RA patients	Increased risk: - preterm delivery (20% in high vs. 12% in low disease activity) - SGA infants (13% vs. 7%) No increased risk: - cesarean section
Brouwer[36]	Prospective	162 female RA patients versus general Dutch population	No increased risk: - miscarriage

Long-term effects on the offspring

Lower birth weight (even within the normal range) has been associated with an increased risk of cardiovascular and metabolic disease in adulthood[48, 49]. This effect is even more prominent if the children display rapid catch-up growth in weight during their first year of life. De Steenwinkel et al.[50] examined growth charts of 167 children born to women with RA. Of these children, 28% expressed rapid catch-up growth in weight, which was associated with maternal disease activity. A selection of 108 of these children was again evaluated at around 7[51]. At that age, these children did not have a high-risk profile in anthropometric measures, such as an increased blood pressure or altered body composition compared with children born to healthy mothers.

Maternal use of prednisone during pregnancy has been associated with slightly higher daytime cortisol levels in children of age 7[51]. The clinical consequence of this is not clear, as this observation was not associated with clinical signs of a higher cortisol level.

In conclusion, pregnancy outcome in patients with RA is only slightly impaired, but it is not of clinical relevance to the individual patient, especially when disease activity is well controlled. This is reassuring for patients with RA wishing to conceive in terms of the impact of (well-controlled) RA on pregnancy outcome.

PATHOGENIC MECHANISM UNDERLYING THE IMPROVEMENT OF RA DURING PREGNANCY AND THE FLARE AFTER DELIVERY

The pregnancy-associated improvement and postpartum exacerbation of RA might be the result of multiple hormonal and immunological changes during pregnancy and the gradual return of these changes to prepregnancy values after delivery[52]. Although multiple factors have been investigated, the exact mechanisms behind these phenomena remain unknown.

Hormones in pregnancy and RA

Pregnancy is associated with changes in cortisol, estrogen and progesterone levels. Cortisol was initially believed to be associated with the improvement of disease activity during pregnancy in women with RA, due to its anti-inflammatory effects[53, 54]. Cortisol concentrations rise during pregnancy up to the third trimester and decrease after delivery[54-56]. However, no clear associations were found between cortisol levels and the improvement of RA during pregnancy[54, 57, 58].

As RA is more prevalent in women[59], sex hormones (estrogen and progesterone) were thought to play a role in the pathogenesis and therefore also in the improvement of RA during pregnancy, due to their effects on the immune system[52, 60-64]. In contrast to cortisol, the individual influence of the levels of sex hormones on disease activity has not been studied directly in cohorts of female RA patients.

Autoantibodies

Besides being biomarkers for RA, the RA-associated ACPA and RF are considered to play a role in the pathogenesis of RA as well. Levels of ACPA and RF do not seem to be influenced by pregnancy in autoantibody-positive patients[35, 65]. However, de Man et al.[35] did demonstrate that, during pregnancy, patients without autoantibodies (negative for both ACPA and RF) are more likely to improve than are patients positive for either or both (75% vs. 39%, $p = 0.01$). In addition, in a small cohort study by Forger et al.[65], patients with active disease (DAS28-CRP(3) ≥ 3.2) before and during pregnancy had higher ACPA levels than did patients with low disease activity (DAS28-CRP(3) < 3.2).

Glycosylation

Glycosylation is the process of attachment of sugar molecules. Among others, its important functions include protein folding and receptor binding[66], occurring in roughly 50% of proteins.

Glycosylation studies in patients with RA have mainly focused on the glycosylation of the fragment crystallizable (Fc) region of immunoglobulin G (IgG). In this respect, glycosylation is divided into galactosylation, sialylation, fucosylation, mannosylation, and the presence of bisecting N-acetylglucosamine (GlcNAc)[67, 68]. Nonpregnant patients with RA have a lower percentage of galactosylated IgG molecules than women without RA do, which is found to be correlated with increased disease activity[69] and with a more progressive disease course[70]. During pregnancy the percentages of galactosylated IgG molecules increase. This increase is associated with the improvement of RA disease activity during pregnancy[67, 71, 72]. The correlations that Alavi et al.[72] found in a small study with 23 patients were confirmed by van de Geijn et al.[67] in a larger cohort study.

As each galactose residue can bind one sialic acid residue, IgG molecules lacking a terminal galactose also lack terminal sialic acid residues[67, 71]. However, Bondt et al.[71] showed that the association between increased galactosylation and pregnancy-related RA disease improvement is independent of sialylation.

To date, glycosylation of the Fc region has been studied most extensively[67], regarding the improvement of RA during pregnancy. Nevertheless, both the Fc region and the Fab region of immunoglobulins can be glycosylated. In addition, Ruhaak et al.[66] report changes in the glycosylation of other proteins during pregnancy. This could indicate changes in other molecules that play a role in the pathogenesis of RA could also be responsible for the improvement of RA during pregnancy.

Human leukocyte antigen disparity

To induce tolerance against the semi-allogenic fetus, the maternal immune system is suppressed during pregnancy[73]. It has been hypothesized that the more mother and fetus differ genetically, the more the immune system needs to be suppressed and therefore the more autoimmune diseases like RA should improve[74].

In line with this hypothesis, it has been reported that a high degree of maternal-fetal human leukocyte antigen (HLA) class II disparity in the *DRB1*, *DQA* and *DQB* alleles is associated with improvement of RA disease activity during pregnancy[33, 75, 76]. However, another study failed to reconfirm these data[77].

T cells in RA and pregnancy

During pregnancy, an increase in regulatory T cells (Tregs) and a shift in T helper (Th) cells producing pro-inflammatory type 1 cytokines (Th1) to a profile with Th cells producing anti-inflammatory type 2 cytokines (Th2) can be observed[78, 79]. These shifts are thought necessary to prevent maternal rejection of the semi-allogenic fetus[80]. In parallel with these shifts, an improvement of RA during pregnancy has been described[78, 79].

Other factors in the improvement of RA

An increase during pregnancy in the anti-inflammatory cytokines interleukin-1 receptor antagonist (IL1Ra) and soluble tumor necrosis factor receptors (sTNFR) have been implicated in the improvement of RA during pregnancy, among other factors[81]. Furthermore, a decrease in neutrophil function has been hypothesized to, at least partially, underlie the improvement of RA during pregnancy[82]. Further, the levels of mannose-binding lectin (MBL) were hypothesized to be related to a favorable RA disease course during pregnancy[83]. However, in a cohort of 216 patients, van de Geijn et al.[84] found no associations between MBL and changes in RA disease activity during the gestational period .

Post-partum deterioration of RA

As mentioned before, patients with RA are at an increased risk of a flare in disease activity after delivery. As changes in hormone levels and alterations in the immune system are considered to be responsible for the pregnancy-related improvement of RA, the postpartum flares can be explained by the return of the immune system to the preconception state[64]. Another theory on the postpartum deterioration involves the high levels of prolactin in breastfeeding women. Prolactin, a peptide hormone, has pro-inflammatory effects such as enhanced B-cell generation, cell proliferation, development of antigen-presenting cells, immunoglobulin production and upregulation of Th1 type cytokines (IL-1, IL-6, and IL-12)[85]. In their prospective cohort from late pregnancy and onwards, Barrett et al.[86] reported that breastfeeding is associated with increased disease activity at 6 months postpartum based

on tender and swollen joint counts, CRP levels, and self-reported symptoms of pain and swelling. Although this could be an effect of prolactin, the results need to be interpreted with caution, as women who breastfed probably do not use medication and are therefore more prone to deteriorate during the lactation period[64, 86].

In conclusion, most likely, the improvement of RA during pregnancy can be attributed not only to one underlying mechanism but also to changes in several factors, which together cause subtle changes in the maternal immune response resulting in tolerance towards the fetus and a decrease in RA disease activity.

MEDICATION AND PREGNANCY

Disease-modifying antirheumatic drugs that can be prescribed during pregnancy

Of the traditional disease-modifying antirheumatic drugs (DMARDs), sulfasalazine (SSZ) and hydroxychloroquine are most frequently used during pregnancy in RA, due to their known efficacy in RA and proven safety during pregnancy.

Several studies have shown that SSZ can be used safely during pregnancy, although SSZ crosses the placenta[87]. As some case reports have noted the occurrence of aplastic anaemia and neutropenia in children born to women who used > 2 g SSZ daily, it is recommended not to exceed this dose in pregnant women[88, 89]. SSZ inhibits the gastrointestinal and cellular uptake of folate; therefore, folate supplementation should be prescribed throughout the entire pregnancy. Only trace amounts of uncleaved SSZ have been found in breast milk, but sulphapyridine (a metabolite of SSZ) levels can be found in breast milk at 30-60% of maternal serum levels. Nevertheless breastfeeding is considered safe in the in the healthy, full-term infant. In case of dysmaturity, prematurity, hyperbilirubinemia, ill-health, or infants with a glucose-6-phosphate dehydrogenase deficiency, breastfeeding when using SSZ should be avoided[87].

Hydroxychloroquine crosses the placenta, but no increase in congenital malformations was reported in several hundreds of pregnancies at the recommended daily dose of 200-400 mg. Moreover, long-term follow up studies of children did not reveal visual, hearing, or developmental abnormalities[87]. Hydroxychloroquine can only be detected in trace amounts in breastmilk and is therefore considered compatible with breastfeeding[87].

Of the less frequently prescribed DMARDs, both azathioprine and cyclosporine are considered safe during pregnancy, but they are hardly prescribed in RA pregnancies due to a less favourable balance between efficacy and safety[87].

DMARDs that should be avoided during pregnancy

As MTX is known to be teratogenic and to induce miscarriages, it should be avoided during pregnancy[87, 90]. In general, it is advised to stop MTX 3 months before a planned pregnancy[91]; however, recent evidence suggests that a shorter period might also be appropriate[90]. MTX can be detected in small amounts in breastmilk and should be stopped because of a theoretical risk[87].

Leflunomide is a known teratogen in animal studies. Human data are less clear, with some studies reporting no excess of congenital malformations after first-trimester exposure to leflunomide followed by a washout procedure with cholestyramine for enhanced elimination from the body. Nevertheless, according to expert opinion, leflunomide should be discontinued before a pregnancy and a wash out procedure should be performed until the levels are undetectable[87].

Nonsteroidal anti-inflammatory drugs

First- and second-trimester exposure to NSAIDs is not teratogenic. However, the use of NSAIDs has been associated with an increase in miscarriages and a prolonged TTP[9, 87]. After week 20 of gestation, all NSAIDs can impair renal function and cause constriction of the ductus arteriosus. The latter risk increases with gestational age. Therefore, and as NSAID are known to impair labor, all NSAIDs should be discontinued before the 32nd gestational week. NSAIDs can only be detected in low concentrations in breastmilk and are therefore considered compatible with breastfeeding. Data on the safety of cyclooxygenase (COX) inhibitors during pregnancy are scarce; therefore, it is sometimes recommended to switch to nonselective COX inhibitors during pregnancy[87]. Nonselective COX inhibitors can be combined with omeprazole during pregnancy[92].

Glucocorticosteroids

Non-fluorinated glucocorticoids, such as prednisone, are metabolized by the placenta; therefore, no more than 10% of the drug reaches the fetus. Although the association between the use of prednisone during pregnancy and an increase of oral clefts is controversial, the use of prednisone is not considered to be teratogenic in general[87, 93]. During pregnancy, glucocorticoids may exert the same side effects as outside of pregnancy, which might be more damaging during pregnancy, like insulin resistance. Furthermore, of relevance for pregnancy, the use of corticosteroids has been associated with intrauterine growth restriction, shorter gestational age[43], premature rupture of the membranes, and longer TTP[9, 87, 93].

Fluorinated glucocorticosteroids are not inactivated by the placenta. Their use during pregnancy should be restricted to fetal indications, for example, the induction of fetal lung maturation[87].

TNF inhibitors and pregnancy

Increasingly more data are available on the use of TNF inhibitors during pregnancy. As the majority of reports involved unplanned pregnancies initially, most data concern exposure during the first trimester of pregnancy. More recent reports include intentional treatment of pregnant women until the end of pregnancy. These reports mainly involve women with inflammatory bowel disease (IBD), as adequate control of maternal disease activity in IBD might even be more important than in women with arthritis. Most data are on infliximab and adalimumab, as these were the first TNF inhibitors registered for both arthritis and IBD. Data on the use of etanercept and certolizumab during pregnancy are increasing, whereas pregnancy data on golimumab are still limited[94-96].

The use of TNF inhibitors has not been associated with congenital abnormalities or adverse pregnancy outcome[94, 95]. However, very recently a prospective study from the European Network of Teratology Information Services on 495 pregnancies that were exposed to TNF inhibitors during first trimester and 1532 pregnancies from healthy pregnant women revealed a small increase in major birth defects (5% vs. 1,5%; OR 2.2 95% CI 1.0-4.8). These results are not easy to interpret since no distinct pattern of malformations could be identified which would be expected for typical teratogens. In addition, the rate of 1.5% major birth defects in the comparison cohort is lower than the prevalence of all non-chromosomal anomalies of 2.2% recorded by EUROCAT[97].

TNF inhibitors that contain an Fc part of an IgG molecule are actively transported from the maternal to the fetal circulation starting at around week 18 of gestation[98]. This transport is mediated through binding of the Fc part to the fetal Fc receptor. Both infliximab and adalimumab bind to this receptor with high affinity. After third-trimester exposure, the serum levels of infliximab and adalimumab are up to 3 times higher in the newborn than in the mother. Serum levels only gradually reduce over time, and they can still be detected in the newborn 6 months after birth[99]. The limited data suggest that placental transport might not be a major concern for etanercept[100, 101]. For golimumab no data are available. Certolizumab has a pegylated Fab prime component; hence, it is not actively transported over the placenta as it lacks an Fc tail. After third-trimester exposure to certolizumab, only trace amounts can be detected in the fetal circulation[99].

Small studies indicate no increase of infections during the first year of life of newborns after intrauterine exposure to TNF inhibitors, normal response to vaccination, and developmental milestones achieved in a timely manner[96]. In contrast to these reassuring data, a small case series describes the presence of transient severe neutropenia, subsequently complicated by skin infections, in 4 newborns after intrauterine exposure to infliximab (including the third trimester)[102]. Furthermore, in view of the death of a 4.5-month-old female infant who developed disseminated Bacillus Calmette-Guérin (BCG) following BCG vaccination at 3

months of age, live vaccines should be considered with caution. Her mother had been treated for Crohn's disease throughout her pregnancy with infliximab[103]. It is therefore advised that, after intrauterine exposure to TNF inhibitors (with the exception of certolizumab), all live vaccines should be postponed to 6 months of age[94].

THE MALE PERSPECTIVE

There are no well executed studies available on the fertility of male patients with RA and the impact of their disease on the pregnancy outcome of their partners. Lower testosterone levels have been described in male patients with RA than in healthy controls[104], but whether this also results in less fertility is not known. In addition, little is known on the effects of antirheumatic drugs and medication.

Treatment with SSZ has been associated with oligospermia, reduced sperm motility, an increased proportion of abnormal forms, and infertility. Spermatogenesis usually recovers 2 months after withdrawal of the drug[105]. An expert publication advised discontinued MTX in men 3 months before a planned pregnancy, although it also states the lack of an underlying literature basis[91]. Recent studies on a total of 162 pregnancies suggest that paternal exposure to low-dose MTX at time of conception is not associated with an excess risk of birth defects[106, 107]. In general, low-dose MTX treatment is not considered to be associated with oligospermia, motility, concentration, and morphology in semen, although oligospermia and reversible sterility have been observed in case reports[108]. Theoretically, glucocorticosteroids may impair male fertility[109], but clinical studies are lacking. One study suggests that the chronic use of NSAIDs may also impair sperm quality[110]. The previous literature, based on relatively small patient numbers, suggested that TNF inhibitors may negatively impact sperm quality[111, 112]. More recent data show that TNF inhibitors do not have a negative impact on the quality of semen or male fertility, nor do they increase the risk of adverse pregnancy outcome[113-115]. In vitro studies suggest that chloroquine may negatively impair sperm motility. No clinical data are available on chloroquine and hydroxychloroquine[108].

PRACTICAL APPROACH TO RA PATIENTS WHO WISH TO CONCEIVE

The female patient

RA patients who wish to conceive should consult both a rheumatologist and a gynecologist in a timely manner. Given the restrictions of pregnancy, medication should be adjusted in time and one should strive for low disease activity or remission whenever possible. In general, all our RA patients hoping to conceive were prescribed SSZ and HCQ. If this did not result in low disease activity, prednisone preferably not higher than 7.5 mg daily and/or a TNF inhibitor was added. In patients already on a TNF inhibitor, the indication for the TNF inhibitor is reevaluated, but most often continued, as discontinuation TNF inhibitors usually results in high disease activity. Women with RA might be physically impaired; in such cases, a pregnancy might have a higher impact on those women than on other patients. This should be anticipated. A frequent concern of women with RA is that their children have a considerable risk of developing this disease as well. In this respect, our patients may be assured: although there is some increased relative risk, given the relatively low prevalence of RA of 1%, the absolute risk is still low.

In general the following check list can be used:

- Age of patient
- Social context (social roles and work)
- Number and course of previous pregnancies. What was the time to pregnancy in previous pregnancies? Did the couple require assisted reproductive techniques? Were previous pregnancies of the same partner?
- How is her menstrual cycle? When using oral contraceptives: how was her menstrual cycle before starting the oral contraceptives.
- Family history. Any genetically determined diseases in her family?
- Regarding her partner: does he have a chronic illness; does he use medication, alcohol, or drugs, or does he smoke? Medical history, diseases that might affect fertility (cryptorchidism?). Are there any genetically determined diseases in his family?
- Current medication, disease activity, extra-articular features?
- Functional impairment
- Smoking, alcohol, drugs
- Comorbidity
- Sexual problems

The male patient

In general, the same checklist can be used for male patients, with the relevant adaptations. In addition, low disease activity and preferably remission should be the goal when treating male patients. For this purpose, we prescribe a low dose of prednisone sometimes in combination with hydroxychloroquine or a TNF inhibitor. MTX seems to be a good option for male patients who wish to conceive; nevertheless we are still cautious in prescribing it in this particular situation. For some medications, like MTX and TNF inhibitors, no impact on sperm quality has been described at a group level, whereas an association between the prescription of a certain drug and impaired sperm quality has been suggested in case reports

or small studies[108, 111, 112]. In these particular cases, one may consider referring the patient for a sperm analysis. Except for using cyclophosphamide, we do not advise cryopreservation for male RA patients before the start of medication.

SUMMARY

Fertility is impaired in female patients with RA; > 40% of patients with RA need > 1 year to conceive. Higher disease activity, the use of NSAIDs, and the use of prednisone > 7.5 mg daily are associated with a longer time to pregnancy in patients with RA. During pregnancy, disease activity usually improves, although less than previously thought; > 50% of pregnant patients with RA still experience moderate to high disease activity during the third trimester. The improvement of RA during pregnancy can be attributed to not just a single mechanism but most likely several different pathogenic mechanisms.

Almost all pregnancy complications are slightly increased in patients with RA, especially if the disease is active. The clinical relevance for the individual patient is doubtful, especially if the disease is well controlled.

The challenges encountered by male patients with RA who wish to conceive have not been investigated as much.

Although the use of medication is restricted during pregnancy, there are far more treatment options both for women and men than generally thought. Increasing evidence is available on the safe use of TNF inhibitors in both female and male RA patients who wish to conceive.

As prolonged periods of uncontrolled disease activity are known to be harmful for the patient and given the known impact of high disease activity on fertility and pregnancy outcome, a treat-to-target approach is advocated in both in female and male RA patients wishing to conceive.

REFERENCES

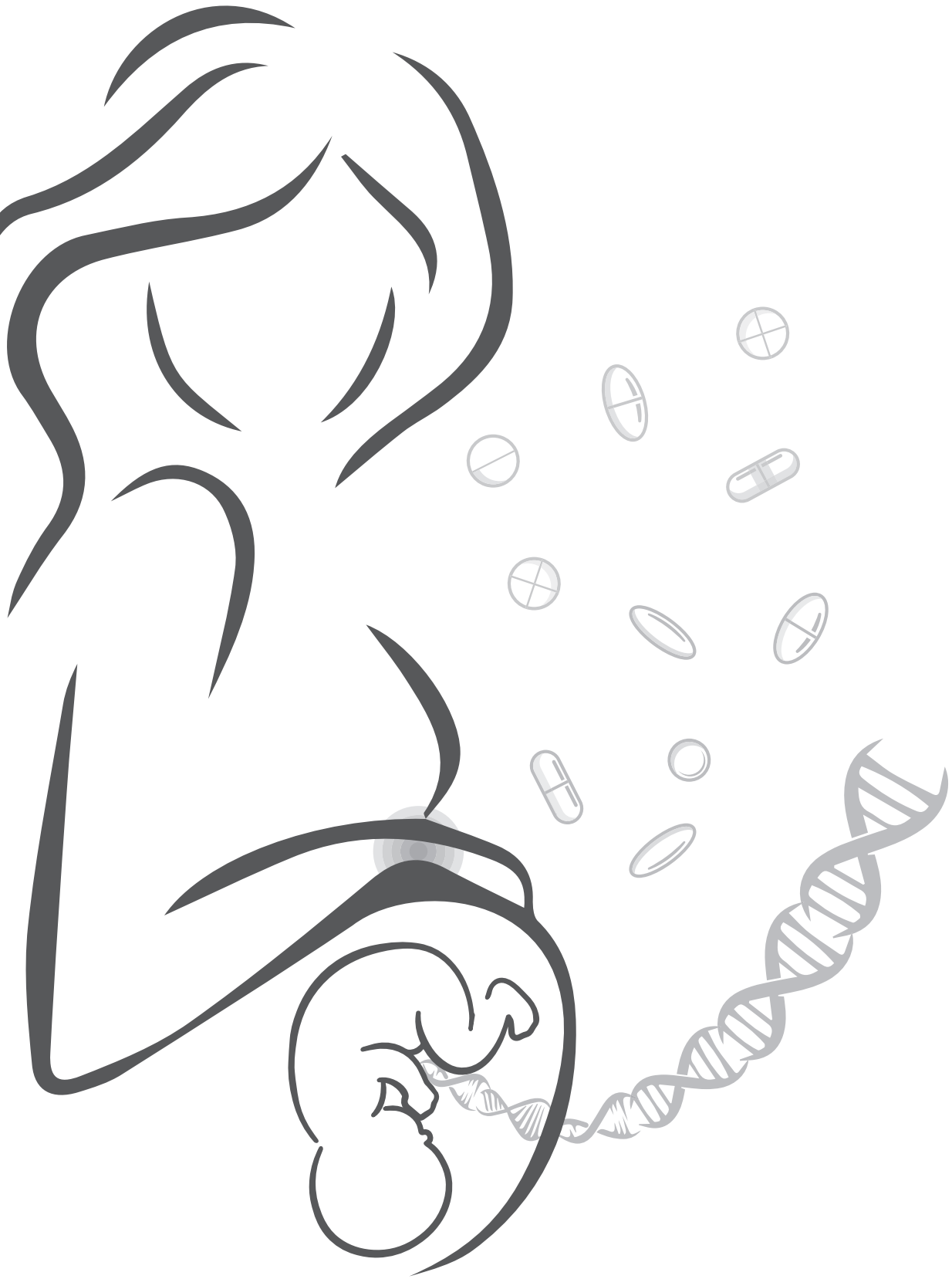
1. Hargreaves ER. A survey of rheumatoid arthritis in West Cornwall; a report to the Empire Rheumatism Council. Ann Rheum Dis 1958;17:61-75.
2. Kay A, Bach F. Subfertility before and after the Development of Rheumatoid Arthritis in Women. Ann Rheum Dis 1965;24:169-73.
3. Wallenius M, Skomsvoll JF, Irgens LM, Salvesen KA, Nordvag BY, Koldingsnes W, et al. Fertility in women with chronic inflammatory arthritides. Rheumatology (Oxford) 2011;50:1162-7.
4. Wallenius M, Skomsvoll JF, Irgens LM, Salvesen KA, Nordvag BY, Koldingsnes W, et al. Parity in patients with chronic inflammatory arthritides childless at time of diagnosis. Scand J Rheumatol 2012;41:202-7.
5. Skomsvoll JF, Ostensen M, Baste V, Irgens LM. Number of births, interpregnancy interval, and subsequent pregnancy rate after a diagnosis of inflammatory rheumatic disease in Norwegian women. J Rheumatol 2001;28:2310-4.
6. Del Junco DJ, Annegers JF, Coulam CB, Luthra HS. The relationship between rheumatoid arthritis and reproductive function. Br J Rheumatol 1989;28 Suppl 1:33; discussion 42-5.
7. Jawaheer D, Zhu JL, Nohr EA, Olsen J. Time to pregnancy among women with rheumatoid arthritis. Arthritis Rheum 2011;63:1517-21.
8. Clowse ME, Chakravarty E, Costenbader KH, Chambers C, Michaud K. Effects of infertility, pregnancy loss, and patient concerns on family size of women with rheumatoid arthritis and systemic lupus erythematosus. Arthritis Care Res (Hoboken) 2012;64:668-74.
9. Brouwer J, Hazes JM, Laven JS, Dolhain RJ. Fertility in women with rheumatoid arthritis: influence of disease activity and medication. Ann Rheum Dis 2014;in press.
10. Juul S, Karmaus W, Olsen J. Regional differences in waiting time to pregnancy: pregnancy-based surveys from Denmark, France, Germany, Italy and Sweden. The European Infertility and Subfecundity Study Group. Hum Reprod 1999;14:1250-4.
11. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod 2007;22:1506-12.
12. Provost M, Eaton JL, Clowse ME. Fertility and infertility in rheumatoid arthritis. Curr Opin Rheumatol 2014;26:308-14.
13. Brouwer J, Laven JS, Hazes JM, Schipper I, Dolhain RJ. Levels of serum anti-Mullerian hormone, a marker for ovarian reserve, in women with rheumatoid arthritis. Arthritis Care Res (Hoboken) 2013;65:1534-8.
14. Saketos M, Sharma N, Santoro NF. Suppression of the hypothalamic-pituitary-ovarian axis in normal women by glucocorticoids. Biol Reprod 1993;49:1270-6.
15. Whirledge S, Cidlowski JA. A role for glucocorticoids in stress-impaired reproduction: beyond the hypothalamus and pituitary. Endocrinology 2013;154:4450-68.
16. van den Broe NR, Letsky EA. Pregnancy and the erythrocyte sedimentation rate. BJOG 2001;108:1164-7.
17. de Man YA, Hazes JM, van de Geijn FE, Krommenhoek C, Dolhain RJ. Measuring disease activity and functionality during pregnancy in patients with rheumatoid arthritis. Arthritis Rheum 2007;57:716-22.
18. Ostensen M, Husby G. A prospective clinical study of the effect of pregnancy on rheumatoid arthritis and ankylosing spondylitis. Arthritis Rheum 1983;26:1155-9.
19. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44-8.
20. de Man YA, Dolhain RJ, Hazes JM. Disease activity or remission of rheumatoid arthritis before, during and following pregnancy. Curr Opin Rheumatol 2014;26:329-33.
21. Barrett JH, Brennan P, Fiddler M, Silman AJ. Does rheumatoid arthritis remit during pregnancy and relapse postpartum? Results from a nationwide study in the United Kingdom performed prospectively from late pregnancy. Arthritis Rheum 1999;42:1219-27.
22. Hench PS. The ameliorating effect of pregnancy on chronic atrophic (infectious rheumatoid) arthritis, fibrositis and intermittent hydrarthritis. Mayo Clin Proc 1938;13:161-7.

23. Lewis-Faning E. Report on an enquiry into the aetiological factors associated with rheumatoid arthritis. Ann Rheum Dis 1950;9 (Suppl).
24. Torrent PB, Marcet CA. Effect of pregnancy on the course of chronic progressive polyarthritis. Rev Esp Reum Enferm Osteoartic 1951;4:96-9.
25. Oka M. Effect of pregnancy on the onset and course of rheumatoid arthritis. Ann Rheum Dis 1953;12:227-9.
26. Betson JR, Jr., Dorn RV. Forty Cases of Arthritis and Pregnancy. J Int Coll Surg 1964;42:521-6.
27. Morris WI. Pregnancy in rheumatoid arthritis and systemic lupus erythematosus. Aust N Z J Obstet Gynaecol 1969;9:136-44.
28. Neely NT, Persellin RH. Activity of rheumatoid arthritis during pregnancy. Tex Med 1977;73:59-63.
29. Ostensen M, Aune B, Husby G. Effect of pregnancy and hormonal changes on the activity of rheumatoid arthritis. Scand J Rheumatol 1983;12:69-72.
30. Klipple GL, Cecere FA. Rheumatoid arthritis and pregnancy. Rheum Dis Clin North Am 1989;15:213-39.
31. Smith WD, West HF. Pregnancy and rheumatoid arthritis. Acta Rheumatol Scand 1960;6:189-201.
32. Unger A, Kay A, Griffin AJ, Panayi GS. Disease activity and pregnancy associated alpha 2-glycoprotein in rheumatoid arthritis during pregnancy. Br Med J (Clin Res Ed) 1983;286:750-2.
33. Nelson JL, Hughes KA, Smith AG, Nisperos BB, Branchaud AM, Hansen JA. Maternal-fetal disparity in HLA class II alloantigens and the pregnancy-induced amelioration of rheumatoid arthritis. N Engl J Med 1993;329:466-71.
34. de Man YA, Dolhain RJ, van de Geijn FE, Willemsen SP, Hazes JM. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum 2008;59:1241-8.
35. de Man YA, Bakker-Jonges LE, Goorbergh CM, Tillemans SP, Hooijkaas H, Hazes JM, et al. Women with rheumatoid arthritis negative for anti-cyclic citrullinated peptide and rheumatoid factor are more likely to improve during pregnancy, whereas in autoantibody-positive women autoantibody levels are not influenced by pregnancy. Ann Rheum Dis 2010;69:420-3.
36. Brouwer J, Laven JS, Hazes JM, Dolhain RJ. Brief Report: Miscarriages in Female Rheumatoid Arthritis Patients: Associations With Serologic Findings, Disease Activity, and Antirheumatic Drug Treatment. Arthritis Rheumatol 2015;67:1738-43.
37. Skomsvoll JF, Ostensen M, Irgens LM, Baste V. Perinatal outcome in pregnancies of women with connective tissue disease and inflammatory rheumatic disease in Norway. Scand J Rheumatol 1999;28:352-6.
38. Norgaard M, Larsson H, Pedersen L, Granath F, Askling J, Kieler H, et al. Rheumatoid arthritis and birth outcomes: a Danish and Swedish nationwide prevalence study. J Intern Med 2010;268:329-37.
39. Lin HC, Chen SF, Lin HC, Chen YH. Increased risk of adverse pregnancy outcomes in women with rheumatoid arthritis: a nationwide population-based study. Ann Rheum Dis 2010;69:715-7.
40. Bharti B, Lee SJ, Lindsay SP, Wingard DL, Jones KL, Lemus H, et al. Disease Severity and Pregnancy Outcomes in Women with Rheumatoid Arthritis: Results from the Organization of Teratology Information Specialists Autoimmune Diseases in Pregnancy Project. J Rheumatol 2015;in press.
41. Wolfberg AJ, Lee-Parritz A, Peller AJ, Lieberman ES. Association of rheumatologic disease with preeclampsia. Obstet Gynecol 2004;103:1190-3.
42. Reed SD, Vollan TA, Svec MA. Pregnancy outcomes in women with rheumatoid arthritis in Washington State. Matern Child Health J 2006;10:361-6.
43. de Man YA, Hazes JM, van der Heide H, Willemsen SP, de Groot CJ, Steegers EA, et al. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: results of a national prospective study. Arthritis Rheum 2009;60:3196-206.
44. Nelson JL, Voigt LF, Koepsell TD, Dugowson CE, Daling JR. Pregnancy outcome in women with rheumatoid arthritis before disease onset. J Rheumatol 1992;19:18-21.
45. Langen ES, Chakravarty EF, Liaquat M, El-Sayed YY, Druzin ML. High rate of preterm birth in pregnancies complicated by rheumatoid arthritis. Am J Perinatol 2014;31:9-14.
46. Wallenius M, Salvesen KA, Daltveit AK, Skomsvoll JF. Rheumatoid arthritis and outcomes in first and subsequent births based on data from a national birth registry. Acta Obstet Gynecol Scand 2014;93:302-7.
47. Rom AL, Wu CS, Olsen J, Kjaergaard H, Jawaheer D, Hetland ML, et al. Fetal growth and preterm birth in children exposed to maternal or paternal rheumatoid arthritis: a nationwide cohort study. Arthritis Rheumatol 2014;66:3265-73.

48. Ong KK, Dunger DB. Perinatal growth failure: the road to obesity, insulin resistance and cardiovascular disease in adults. Best Pract Res Clin Endocrinol Metab 2002;16:191-207.
49. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. JAMA 2009;301:2234-42.
50. de Steenwinkel FD, Hokken-Koelega AC, de Ridder MA, Hazes JM, Dolhain RJ. Rheumatoid arthritis during pregnancy and postnatal catch-up growth in the offspring. Arthritis Rheumatol 2014;66:1705-11.
51. de Steenwinkel FD, Hokken-Koelega AC, Hazes JM, Dolhain RJ. The influence of foetal prednisone exposure on the cortisol levels in the offspring. Clin Endocrinol (Oxf) 2014;80:804-10.
52. Ostensen M, Villiger PM. The remission of rheumatoid arthritis during pregnancy. Semin Immunopathol 2007;29:185-91.
53. Masi AT, Feigenbaum SL, Chatterton RT. Hormonal and pregnancy relationships to rheumatoid arthritis: convergent effects with immunologic and microvascular systems. Semin Arthritis Rheum 1995;25:1-27.
54. Elenkov IJ, Hoffman J, Wilder RL. Does differential neuroendocrine control of cytokine production govern the expression of autoimmune diseases in pregnancy and the postpartum period? Mol Med Today 1997;3:379-83.
55. D'Anna-Hernandez KL, Ross RG, Natvig CL, Laudenslager ML. Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol. Physiol Behav 2011;104:348-53.
56. Nolten WE, Rueckert PA. Elevated free cortisol index in pregnancy: possible regulatory mechanisms. Am J Obstet Gynecol 1981;139:492-8.
57. Oka M. Activity of rheumatoid arthritis and plasma 17-hydroxycorticosteroids during pregnancy and following parturition: report on two cases. Acta Rheumatol Scand 1958;4:243-8.
58. Ostensen M. Glucocorticosteroids in pregnant patients with rheumatoid arthritis. Z Rheumatol 2000;59 Suppl 2:II/70-4.
59. Whitacre CC. Sex differences in autoimmune disease. Nat Immunol 2001;2:777-80.
60. Cutolo M, Sulli A, Seriolo B, Accardo S, Masi AT. Estrogens, the immune response and autoimmunity. Clin Exp Rheumatol 1995;13:217-26.
61. Ostensen M. Sex hormones and pregnancy in rheumatoid arthritis and systemic lupus erythematosus. Ann N Y Acad Sci 1999;876:131-43; discussion 44.
62. Kahn DA, Baltimore D. Pregnancy induces a fetal antigen-specific maternal T regulatory cell response that contributes to tolerance. Proc Natl Acad Sci U S A 2010;107:9299-304.
63. Kanik KS, Wilder RL. Hormonal alterations in rheumatoid arthritis, including the effects of pregnancy. Rheum Dis Clin North Am 2000;26:805-23.
64. Dolhain RJ. Rheumatoid arthritis and pregnancy; not only for rheumatologists interested in female health issues. Ann Rheum Dis 2010;69:317-8.
65. Forger F, Vallbracht I, Helmke K, Villiger PM, Ostensen M. Pregnancy mediated improvement of rheumatoid arthritis. Swiss Med Wkly 2012;142:w13644.
66. Ruhaak LR, Uh HW, Deelder AM, Dolhain RE, Wuhrer M. Total plasma N-glycome changes during pregnancy. J Proteome Res 2014;13:1657-68.
67. van de Geijn FE, Wuhrer M, Selman MH, Willemsen SP, de Man YA, Deelder AM, et al. Immunoglobulin G galactosylation and sialylation are associated with pregnancy-induced improvement of rheumatoid arthritis and the postpartum flare: results from a large prospective cohort study. Arthritis Res Ther 2009;11:R193.
68. Nimmerjahn F, Anthony RM, Ravetch JV. Agalactosylated IgG antibodies depend on cellular Fc receptors for in vivo activity. Proc Natl Acad Sci U S A 2007;104:8433-7.
69. Rook GA, Steele J, Brealey R, Whyte A, Isenberg D, Sumar N, et al. Changes in IgG glycoform levels are associated with remission of arthritis during pregnancy. J Autoimmun 1991;4:779-94.
70. van Zeben D, Rook GA, Hazes JM, Zwinderman AH, Zhang Y, Ghelani S, et al. Early agalactosylation of IgG is associated with a more progressive disease course in patients with rheumatoid arthritis: results of a follow-up study. Br J Rheumatol 1994;33:36-43.
71. Bondt A, Selman MH, Deelder AM, Hazes JM, Willemsen SP, Wuhrer M, et al. Association between galactosylation of immunoglobulin G and improvement of rheumatoid arthritis during pregnancy is independent of sialylation. J Proteome Res 2013;12:4522-31.

72. Alavi A, Arden N, Spector TD, Axford JS. Immunoglobulin G glycosylation and clinical outcome in rheumatoid arthritis during pregnancy. J Rheumatol 2000;27:1379-85.
73. Hazes JM, Coulie PG, Geenen V, Vermeire S, Carbonnel F, Louis E, et al. Rheumatoid arthritis and pregnancy: evolution of disease activity and pathophysiological considerations for drug use. Rheumatology (Oxford) 2011;50:1955-68.
74. Nelson JL, Hansen JA. Autoimmune diseases and HLA. Crit Rev Immunol 1990;10:307-28.
75. van der Horst-Bruinsma IE, de Vries RR, de Buck PD, van Schendel PW, Breedveld FC, Schreuder GM, et al. Influence of HLA-class II incompatibility between mother and fetus on the development and course of rheumatoid arthritis of the mother. Ann Rheum Dis 1998;57:286-90.
76. Zrour SH, Boumiza R, Sakly N, Mannai R, Korbaa W, Younes M, et al. The impact of pregnancy on rheumatoid arthritis outcome: the role of maternofetal HLA class II disparity. Joint Bone Spine 2010;77:36-40.
77. Brennan P, Barrett J, Fiddler M, Thomson W, Payton T, Silman A. Maternal-fetal HLA incompatibility and the course of inflammatory arthritis during pregnancy. J Rheumatol 2000;27:2843-8.
78. Amin S, Peterson EJ, Reed AM, Mueller DL. Pregnancy and rheumatoid arthritis: insights into the immunology of fetal tolerance and control of autoimmunity. Curr Rheumatol Rep 2011;13:449-55.
79. Forger F, Marcoli N, Gadola S, Moller B, Villiger PM, Ostensen M. Pregnancy induces numerical and functional changes of CD4+CD25 high regulatory T cells in patients with rheumatoid arthritis. Ann Rheum Dis 2008;67:984-90.
80. Billington WD. The immunological problem of pregnancy: 50 years with the hope of progress. A tribute to Peter Medawar. J Reprod Immunol 2003;60:1-11.
81. Ostensen M, Forger F, Nelson JL, Schuhmacher A, Hebisch G, Villiger PM. Pregnancy in patients with rheumatic disease: anti-inflammatory cytokines increase in pregnancy and decrease post partum. Ann Rheum Dis 2005;64:839-44.
82. Crocker IP, Baker PN, Fletcher J. Neutrophil function in pregnancy and rheumatoid arthritis. Ann Rheum Dis 2000;59:555-64.
83. Garred P, Madsen HO, Marquart H, Hansen TM, Sorensen SF, Petersen J, et al. Two edged role of mannose binding lectin in rheumatoid arthritis: a cross sectional study. J Rheumatol 2000;27:26-34.
84. van de Geijn FE, de Man YA, Wuhrer M, Willemsen SP, Deelder AM, Hazes JM, et al. Mannose-binding lectin does not explain the course and outcome of pregnancy in rheumatoid arthritis. Arthritis Res Ther 2011;13:R10.
85. Orbach H, Shoenfeld Y. Hyperprolactinemia and autoimmune diseases. Autoimmun Rev 2007;6:537-42.
86. Barrett JH, Brennan P, Fiddler M, Silman A. Breast-feeding and postpartum relapse in women with rheumatoid and inflammatory arthritis. Arthritis Rheum 2000;43:1010-5.
87. Ostensen M, Khamashta M, Lockshin M, Parke A, Brucato A, Carp H, et al. Anti-inflammatory and immunosuppressive drugs and reproduction. Arthritis Res Ther 2006;8:209.
88. Zwi LJ, Becroft DM. Intrauterine aplastic anemia and fetal hydrops: a case report. Pediatr Pathol 1986;5:199-205.
89. Levi S, Liberman M, Levi AJ, Bjarnason I. Reversible congenital neutropenia associated with maternal sulphasalazine therapy. Eur J Pediatr 1988;148:174-5.
90. Weber-Schoendorfer C, Chambers C, Wacker E, Beghin D, Bernard N, Network of French Pharmacovigilance C, et al. Pregnancy outcome after methotrexate treatment for rheumatic disease prior to or during early pregnancy: a prospective multicenter cohort study. Arthritis Rheumatol 2014;66:1101-10.
91. Visser K, Katchamart W, Loza E, Martinez-Lopez JA, Salliot C, Trudeau J, et al. Multinational evidence-based recommendations for the use of methotrexate in rheumatic disorders with a focus on rheumatoid arthritis: integrating systematic literature research and expert opinion of a broad international panel of rheumatologists in the 3E Initiative. Ann Rheum Dis 2009;68:1086-93.
92. Matok I, Levy A, Wiznitzer A, Uziel E, Koren G, Gorodischer R. The safety of fetal exposure to proton-pump inhibitors during pregnancy. Dig Dis Sci 2012;57:699-705.
93. Bermas BL. Non-steroidal anti inflammatory drugs, glucocorticoids and disease modifying anti-rheumatic drugs for the management of rheumatoid arthritis before and during pregnancy. Curr Opin Rheumatol 2014;26:334-40.
94. Hyrich KL, Verstappen SM. Biologic therapies and pregnancy: the story so far. Rheumatology (Oxford) 2014;53:1377-85.

95. Chaudrey KH, Kane SV. Safety of Immunomodulators and Anti-TNF Therapy in Pregnancy. Curr Treat Options Gastroenterol 2015;13:77-89.
96. Khan N, Asim H, Lichtenstein GR. Safety of anti-TNF therapy in inflammatory bowel disease during pregnancy. Expert Opin Drug Saf 2014;13:1699-708.
97. Weber-Schoendorfer C, Oppermann M, Wacker E, Bernard N, network of French pharmacovigilance c, Beghin D, et al. Pregnancy outcome after TNF-alpha inhibitor therapy during the first trimester: a prospective multicentre cohort study. Br J Clin Pharmacol 2015;in press.
98. Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H. Evolution of maternofetal transport of immunoglobulins during human pregnancy. Am J Reprod Immunol 1996;36:248-55.
99. Mahadevan U, Wolf DC, Dubinsky M, Cortot A, Lee SD, Siegel CA, et al. Placental transfer of anti-tumor necrosis factor agents in pregnant patients with inflammatory bowel disease. Clin Gastroenterol Hepatol 2013;11:286-92; quiz e24.
100. Murashima A, Watanabe N, Ozawa N, Saito H, Yamaguchi K. Etanercept during pregnancy and lactation in a patient with rheumatoid arthritis: drug levels in maternal serum, cord blood, breast milk and the infant's serum. Ann Rheum Dis 2009;68:1793-4.
101. Berthelsen BG, Fjeldsoe-Nielsen H, Nielsen CT, Hellmuth E. Etanercept concentrations in maternal serum, umbilical cord serum, breast milk and child serum during breastfeeding. Rheumatology (Oxford) 2010;49:2225-7.
102. Guiddir T, Fremond ML, Triki TB, Candon S, Croisille L, Leblanc T, et al. Anti-TNF-alpha therapy may cause neonatal neutropenia. Pediatrics 2014;134:e1189-93.
103. Cheent K, Nolan J, Shariq S, Kiho L, Pal A, Arnold J. Case Report: Fatal case of disseminated BCG infection in an infant born to a mother taking infliximab for Crohn's disease. J Crohns Colitis 2010;4:603-5.
104. Gordon D, Beastall GH, Thomson JA, Sturrock RD. Androgenic status and sexual function in males with rheumatoid arthritis and ankylosing spondylitis. Q J Med 1986;60:671-9.
105. O'Morain C, Smethurst P, Dore CJ, Levi AJ. Reversible male infertility due to sulphasalazine: studies in man and rat. Gut 1984;25:1078-84.
106. Wallenius M, Lie E, Daltveit AK, Salvesen KA, Skomsvoll JF, Kalstad S, et al. No excess risks in offspring with paternal preconception exposure to disease-modifying antirheumatic drugs. Arthritis Rheumatol 2015;67:296-301.
107. Weber-Schoendorfer C, Hoeltzenbein M, Wacker E, Meister R, Schaefer C. No evidence for an increased risk of adverse pregnancy outcome after paternal low-dose methotrexate: an observational cohort study. Rheumatology (Oxford) 2014;53:757-63.
108. Millsop JW, Heller MM, Eliason MJ, Murase JE. Dermatological medication effects on male fertility. Dermatol Ther 2013;26:337-46.
109. Whirledge S, Cidlowski JA. Glucocorticoids, stress, and fertility. Minerva Endocrinol 2010;35:109-25.
110. Martini AC, Molina RI, Tissera AD, Ruiz RD, Fiol de Cuneo M. Analysis of semen from patients chronically treated with low or moderate doses of aspirin-like drugs. Fertil Steril 2003;80:221-2.
111. Mahadevan U, Terdiman JP, Aron J, Jacobsohn S, Turek P. Infliximab and semen quality in men with inflammatory bowel disease. Inflamm Bowel Dis 2005;11:395-9.
112. Montagna GL, Malesci D, Buono R, Valentini G. Asthenoazoospermia in patients receiving anti-tumour necrosis factor {alpha} agents. Ann Rheum Dis 2005;64:1667.
113. Puchner R, Danninger K, Puchner A, Pieringer H. Impact of TNF-blocking agents on male sperm characteristics and pregnancy outcomes in fathers exposed to TNF-blocking agents at time of conception. Clin Exp Rheumatol 2012;30:765-7.
114. Ramonda R, Foresta C, Ortolan A, Bertoldo A, Oliviero F, Lorenzin M, et al. Influence of tumor necrosis factor alpha inhibitors on testicular function and semen in spondyloarthritis patients. Fertil Steril 2014;101:359-65.
115. Micu MC, Micu R, Surd S, Girlovanu M, Bolboaca SD, Ostensen M. TNF-alpha inhibitors do not impair sperm quality in males with ankylosing spondylitis after short-term or long-term treatment. Rheumatology (Oxford) 2014;53:1250-5.



Is disease activity in rheumatoid arthritis during pregnancy and after delivery predictive for disease activity in a subsequent pregnancy?

Hilal Ince-Aşkan
Johanna M.W. Hazes
Radboud J.E.M. Dolhain

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ABSTRACT

Objectives. To determine whether disease activity in women with rheumatoid arthritis (RA) in 1 pregnancy is predictive for disease activity in a subsequent pregnancy.

Methods. In the Pregnancy-induced Amelioration of Rheumatoid Arthritis study, there are prospective data on 27 patients who participated twice. Improvement and deterioration is determined by changes in the Disease Activity Score in 28 joints.

Results. Only 4 patients (14.8%) had comparable disease courses in both pregnancies, whereas treatment remained mostly similar. In contrast, a flare postpartum after the first pregnancy was predictive for a flare after the second pregnancy ($p = 0.003$).

Conclusions. RA disease course in following pregnancies cannot be predicted based upon previous pregnancies. However, a flare postpartum seems to predict subsequent flares.

INTRODUCTION

Pregnancy is the only physiological condition in which rheumatoid arthritis (RA) remits spontaneously in about 50% of cases[1-3]. We previously reported in a prospective cohort study on pregnancy and RA that 48% of patients improved during pregnancy, based upon the Disease Activity Score in 28 joints (DAS28) according to the European League Against Rheumatism (EULAR) response criteria[3].

Some studies showed that improvement in the first pregnancy is predictive for improvement in the next pregnancy[1, 4]. However, in these studies, assessment of the RA disease activity in the first pregnancies was retrospective and self-reported by the women[1].

Understanding the influence of pregnancy and especially the effect of subsequent pregnancies may provide more insight into the pathophysiological mechanisms underlying RA. Further, increased knowledge on the disease course of RA during subsequent pregnancies might also provide valuable information for daily clinical practice.

To our knowledge, the course of RA disease activity has not been prospectively studied during subsequent pregnancies and after delivery. The aim of our present study was to prospectively determine whether RA disease activity course is similar in subsequent pregnancies and after delivery and thereby to determine whether the disease activity course in following pregnancies can be predicted.

MATERIALS AND METHODS

Study population

Our study is embedded in the Pregnancy-induced Amelioration of Rheumatoid Arthritis (PARA) study, a nationwide prospective cohort study from the Netherlands[3]. From May 2002 to August 2008, 475 patients in total who met the 1987 revised criteria of the American College of Rheumatology (ACR)[5] for RA were recruited by their rheumatologist, and from these, 369 were enrolled in the PARA-study. Women were eligible for inclusion if they had a pregnancy wish or were already pregnant (in their first trimester). The PARA-study is believed to be a good representation of the general Dutch pregnant RA population between 2002 and 2008. During the study period, 205 women conceived at least once[6]. There were data available on 27 female patients with RA who had 2 successful pregnancies until delivery resulting in a live birth.

Data collection

Data on disease activity and medication use were collected before conception, if possible, at each trimester during pregnancy (8-12, 18-22 , and 28-32 weeks) and 3 times postpartum(6, 12, and 26 weeks)[3].

Disease activity

DAS28 was calculated based upon the number of swollen joints, the number of tender joints, and the level of C-reactive protein (CRP)[7, 8].

Improvement and deterioration of the disease activity during pregnancy were calculated as differences in DAS28 between first and second, first and third or second and third trimester. Preconception DAS28 was not used in the analysis because this was only available in 6 patients in both pregnancies. Improvement of the disease activity during pregnancy was defined as a decrease in DAS28 > 0.6 and deterioration as an increase in DAS28 > 0.6. Possible disease activity courses were categorized as improvement, deterioration, both improvement and deterioration, and no change. The limit of 0.6 was chosen since it is the lowest meaningful difference according to the EULAR response criteria[3, 9]. In contrast to the EULAR response criteria, an initial DAS28 of ≥ 3.2 was not a prerequisite.

Flares postpartum were calculated as an increase in DAS28 between the third trimester and any of the three time points postpartum of > 0.6.

Statistical analysis

For all subjects, descriptive statistics were calculated as numbers, percentages, means, and standard deviations (SD). A 2-sample Student t test was used to detect differences in mean disease activity scores. Binomial probability tests were used to analyze whether a flare after the first pregnancy was predictive for a subsequent pregnancy. P values < 0.05 were considered statistically significant. Statistical analysis were performed using STATA software version 13.1 for Windows.

Ethics

Our study is in compliance with the Helsinki Declaration. The Medical Ethics Committee at the Erasmus Medical Center Rotterdam, the Netherlands, approved the PARA-study.

RESULTS

Participants

Descriptive statistics of the study population are shown in table 1. In the first and second pregnancies, the mean maternal age was 31.2 years and 33.6 years respectively (p<0.007). Mean duration of RA was 6.5 years in the first pregnancy and 8.7 years in the second pregnancy group. In total, 70.4% of the patients were RF-positive, 55.6% were anti-cyclic citrullinated peptide (anti-CPP)-positive, and 74.1% had erosive RA. General characteristics of the selected patients were comparable with the other patients in the PARA-study (data not shown)[3].

Table 1. Descriptive statistics of study population. Values are n (%) unless otherwise specified.

Characteristics	First Pregnancy, N = 27	Second Pregnancy, N = 27
Age, yrs, mean (SD)*	31.2 (3.6)	33.6 (3.4)
Smoking	3 (11.1)	2 (7.4)
RA duration, yrs, mean (SD)	6.5 (5.6)	8.7 (6.0)
DAS28-CRP(3) first trimester, mean (SD)	3.84 (1.11)	3.81 (1.22)
Patients with moderate and high disease activity, DAS28-CRP(3) ≥ 3.2, at first trimester	19 (70.4)	17 (63.0)
Prednisone use during pregnancy	7 (25.9)	11 (40.7)
SSZ use during pregnancy	5 (18.5)	3 (11.1)
Both prednisone and SSZ use during pregnancy	1 (3.7)	2 (7.4)
No medication during pregnancy	14 (51.9)	11 (40.7)
	In Total	
RF-positive	19 (70.4)	
Anti-CCP-positive	15 (55.6)	
Erosion	20 (74.1)	
MTX use ever	17 (63.0)	

RA: rheumatoid arthritis; DAS 28: Disease Activity Score in 28 joints; CRP: C-reactive protein; SSZ: sulfasalazine; RF: rheumatoid factor; anti-CCP: anti-cyclic citrullinated peptide; MTX: methotrexate.

Disease activity during pregnancy

Mean DAS28 changed during first pregnancy from 3.84 (first trimester) to 3.52 (third trimester) to 3.75 (12 weeks postpartum) and finally 3.82 (26 weeks post-partum). The mean DAS28 during second pregnancy at those timepoints was 3.81, 3.55, 3.74 and 3.41 respectively. In the first pregnancy, in total 37.0% of the patients improved, 22.2% deteriorated, 22.2% both

improved and deteriorated, and 18.5% had no change in disease activity. In the second pregnancy, these numbers were 37.0%, 18.5%,14.8%, and 25.9% respectively (Table 2). Only 4 patients (14.8%) had a comparable disease activity course during their first and second pregnancy, represented by asterisks in table 2.

Table 2. No. of patients in the disease course categories improvement, deterioration, both improvement and deterioration, and no change during first and second pregnancies. Values are n (%).

Disease Activity during Pregnancy	Improvement Second Pregnancy	Deterioration Second Pregnancy	Improvement and Deterioration Second Pregnancy	No Change Second Pregnancy	Total First Pregnancy
Improvement First Pregnancy	2 (7.4)*	3 (11.1)	2 (7.4)	3 (11.1)	10 (37.0)
Deterioration First Pregnancy	4 (14.8)	0 (0)*	0 (0)	2 (7.4)	6 (22.2)
Improvement and Deterioration First pregnancy	2 (7.4)	2 (7.4)	1 (3.7)*	1 (3.7)	6 (22.2)
No change First Pregnancy	2 (7.4)	1 (3.7)	1 (3.7)	1 (3.7)*	5 (18.5)
Total Second Pregnancy	10 (37.0)	6 (22.2)	4 (14.8)	7 (25.9)	27 (100)

* Similar disease courses in both pregnancies

Disease courses in the first and second pregnancies are shown in figure 1.

Medication use during pregnancy

None of the pregnant women received methotrexate (MTX), biologicals, or hydroxychloroquine in the 3 months before conception or during pregnancy. Patients received prednisone, sulfasalazine (SSZ), both prednisone and SSZ, or no medication. In the first and second pregnancies, respectively 14 (51.9%) and 12 patients (44.4%) did not receive any medication. Treatment and also the medication doses were overall similar in 22 patients (81.5%) in both pregnancies.

Flare postpartum

Twenty patients (74.1%) had a flare postpartum after their first pregnancy. In total, 17 patients (63.0%) had a flare after both pregnancies. The occurrence of a flare postpartum after the first pregnancy was predictive for a flare after the second pregnancy (p = 0.003). Three patients (11.1%) had no flare after both pregnancies. Three patients (11.1%) had a flare after their first pregnancy and no flare after their second pregnancy. In 4 patients (14.8%), the RA flared postpartum after their second pregnancy while it did not flare after their first pregnancy. The mean time span between two subsequent pregnancies in patients with and without a flare after the first pregnancy was 924 and 727 days respectively (p = 0.07).

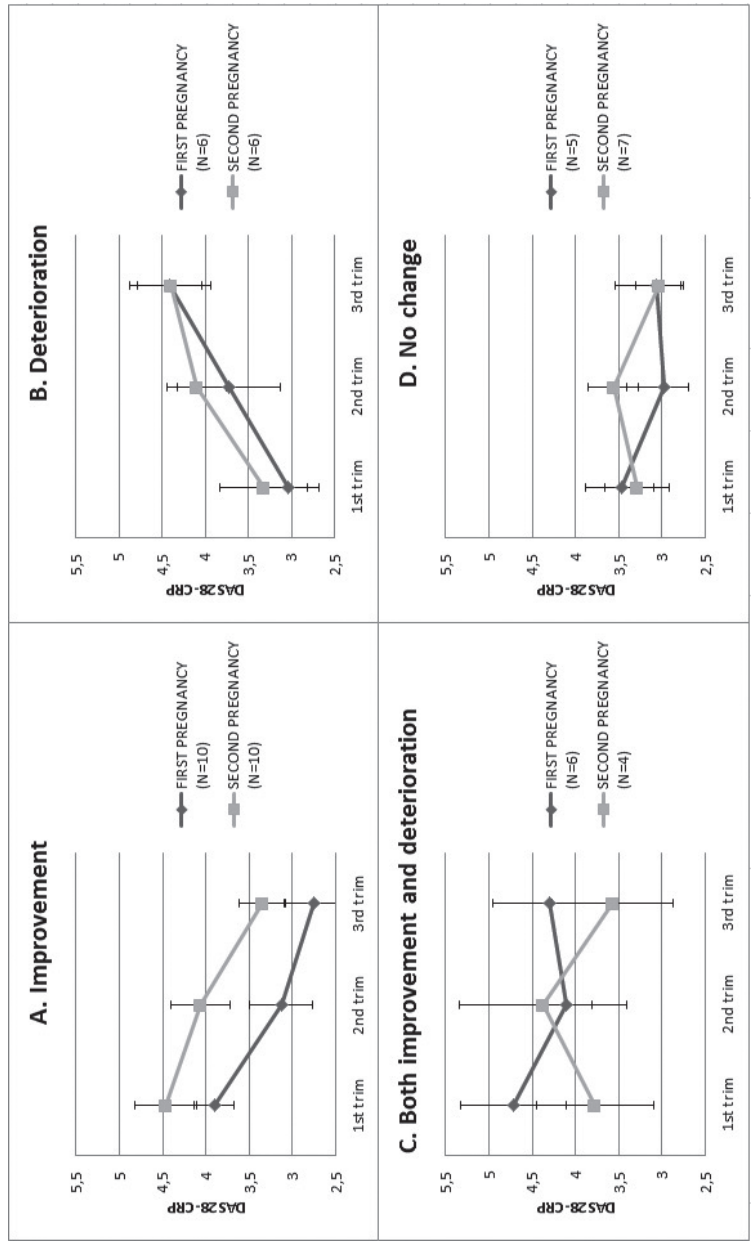


Figure 1. Different disease courses in the first and second pregnancies during the 3 trimesters of pregnancy. On the X-axis, the 3 measurement points during pregnancy are shown. The mean disease activity is depicted on the Y-axis as a mean DAS28-CRP(3) (with standard errors) . The black line represents the first and the gray line the second pregnancies. Please bear in mind that because of the differences in disease course between first and second pregnancies in individual patients, the first and second pregnancies of the same patients are not always allocated to the same subfigure. (A) Improvement in 10 patients in the first and 10 in the second pregnancy. (B) Deterioration in 6 patients in the first and 6 in the second pregnancy. (C) Both improvement and deterioration in 6 patients in the first and 4 patients in the second pregnancy. (D) No change during pregnancy in 5 patients in the first and 7 patients in the second pregnancy.

DAS28: Disease Activity in 28 joints; CRP: C-reactive protein; 1st trim: first trimester of pregnancy; 2nd trim: second trimester of pregnancy; 3rd trim: third trimester of pregnancy.

Medication use postpartum

Eleven patients (40.7%) restarted medication after both pregnancies, which they did not use during pregnancy, within 6 weeks after delivery. At 26 weeks, 18 (66.7%) and 22 patients (81.5%) had started additional medication after their first and second pregnancies, respectively. From these, 10 (37.0%) started with MTX or a combination after their first pregnancy and 14 (51.9%) after their second pregnancy. Overall, treatment postpartum was different in 20 patients (74.1%).

DISCUSSION

To our knowledge, ours is the first prospective study that shows that the disease course in female patients with RA is different in subsequent pregnancies. In our present study, only 14.8% of the patients had a comparable disease activity course while treatment in both pregnancies remained mostly similar. These findings are in contrast to previous studies that claimed that if the disease activity improves during a pregnancy, improvement is likely to occur in subsequent pregnancies as well[1,4]. The most likely explanation for this discrepancy is the fact that in those studies, disease activity was retrospectively determined by self-report.

That the improvement rate in this study is lower (37%) than the 48% reported earlier in the PARA-study[3] is probably the result of using different definitions. In our current study, we chose not to define improvement according to the EULAR response criteria because this would lead to a loss of a considerable number of subjects, since the prerequisite of an initial DAS28 ≥ 3.2. With the EULAR response criteria, 15 out of 35 pregnancies (43%) were classified as improvement. This does not differ from the 48% reported earlier ($p = 0.67$). Seventeen patients (63.0%) had a flare postpartum after both pregnancies. The occurrence of a flare postpartum after the first pregnancy was predictive for a flare after the second pregnancy ($p = 0.003$), despite the differences in treatment between both pregnancies.

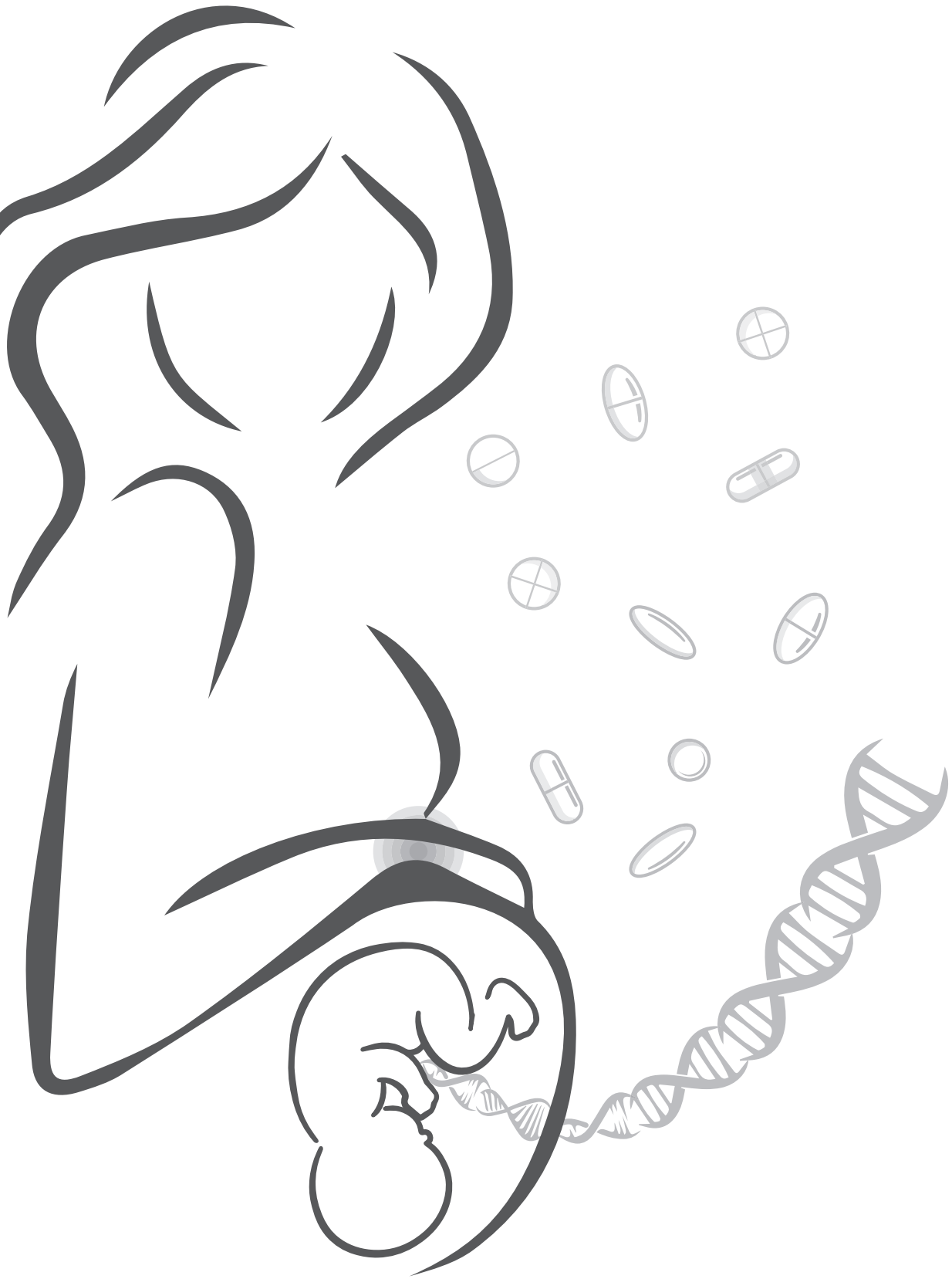
Because we have shown that RA disease activity has a different course during subsequent pregnancies, it is tempting to speculate that fetal-related factors might be more important in the improvement of RA during pregnancy than pure maternal factors. The sex of the children was not associated in our current study with a certain disease activity course (data not shown). Previous studies have shown that incompatibility of human leukocyte antigen (HLA) class II between mother and child seems to positively influence RA disease activity during pregnancy[10-12]. The HLA-related mechanisms were not analyzed because that was beyond the purpose of our article.

Finally, our study has some limitations. First, although in its kind it is a large (predominantly) descriptive study, a study on 27 subjects is still relatively small. Nevertheless, the analysis on postpartum flare reached statistical significance. Second, in the majority, no data on preconception DAS28 were available because some patients were included in their first trimester. However, we have previously reported no significant difference between preconception and first trimester DAS28[6].

Our study shows that we cannot predict the RA disease course in subsequent pregnancies based on the disease course in the first pregnancy. Each pregnant RA patient should be treated to achieve low disease activity without taking into account the disease course of a possible previous pregnancy. However, because the occurrence of flares after delivery in subsequent pregnancies seems to be related to previous flares, this phenomenon can be anticipated both by patients as well as their treating physicians.

REFERENCES

1. Barrett JH, Brennan P, Fiddler M, Silman AJ. Does rheumatoid arthritis remit during pregnancy and relapse postpartum? Results from a nationwide study in the United Kingdom performed prospectively from late pregnancy. Arthritis Rheum 1999;42:1219-27.
2. Ostensen M, Husby G. A prospective clinical study of the effect of pregnancy on rheumatoid arthritis and ankylosing spondylitis. Arthritis Rheum 1983;26:1155-9.
3. de Man YA, Dolhain RJ, van de Geijn FE, Willemsen SP, Hazes JM. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum 2008;59:1241-8.
4. Nelson JL, Ostensen M. Pregnancy and rheumatoid arthritis. Rheum Dis Clin North Am 1997;23:195-212.
5. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
6. Brouwer J, Hazes JM, Laven JS, Dolhain RJ. Fertility in women with rheumatoid arthritis: influence of disease activity and medication. Ann Rheum Dis 2014;in press.
7. de Man YA, Hazes JM, van der Heide H, Willemsen SP, de Groot CJ, Steegers EA, et al. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: results of a national prospective study. Arthritis Rheum 2009;60:3196-206.
8. de Man YA, Hazes JM, van de Geijn FE, Krommenhoek C, Dolhain RJ. Measuring disease activity and functionality during pregnancy in patients with rheumatoid arthritis. Arthritis Rheum 2007;57:716-22.
9. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44-8.
10. van der Horst-Bruinsma IE, de Vries RR, de Buck PD, van Schendel PW, Breedveld FC, Schreuder GM, et al. Influence of HLA-class II incompatibility between mother and fetus on the development and course of rheumatoid arthritis of the mother. Ann Rheum Dis 1998;57:286-90.
11. Nelson JL, Hughes KA, Smith AG, Nisperos BB, Branchaud AM, Hansen JA. Maternal-fetal disparity in HLA class II alloantigens and the pregnancy-induced amelioration of rheumatoid arthritis. N Engl J Med 1993;329:466-71.
12. Zrour SH, Boumiza R, Sakly N, Mannai R, Korbaa W, Younes M, et al. The impact of pregnancy on rheumatoid arthritis outcome: the role of maternofetal HLA class II disparity. Joint Bone Spine 2010;77:36-40.



Identifying clinical factors associated with low disease activity and remission of rheumatoid arthritis during pregnancy

Hilal Ince-Aşkan
Johanna M.W. Hazes
Radboud J.E.M. Dolhain

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ABSTRACT

Objectives. To identify a combination of clinical factors associated with low disease activity and remission in the third trimester during pregnancy in women with rheumatoid arthritis (RA).

Methods. This study is embedded in the Pregnancy-induced Amelioration of Rheumatoid Arthritis (PARA) study, a prospective cohort study. There were data available on 190 pregnancies from first trimester until delivery. Multivariate regression analyses were performed on the disease activity (Disease Activity Score in 28 joints[DAS28] using the C-Reactive Protein[CRP] level) in the third trimester. Independent covariates were the DAS28-CRP(3) in first trimester, prednisone and sulfasalazine use in the first trimester, parity, methotrexate use in past, autoantibody status, the presence of erosions, and RA disease duration.

Results. In multivariate regression models, the DAS28-CRP(3), use of prednisone in the first trimester and the presence of autoantibodies were negatively associated with low disease activity (DAS28-CRP(3) < 3.2) in the third trimester ($P < 0.05$), and the DAS28-CRP(3) and presence of autoantibodies were also associated with remission(DAS28-CRP(3) < 2.6) ($P < 0.001$). Subgroup analysis revealed that the associations of prednisone use and presence of autoantibodies were only present in patients with moderate-to-high disease activity (DAS28-CRP(3) ≥ 3.2) in the first trimester.

Conclusions. RA patients who have a low DAS28-CRP(3) in the first trimester (irrespective of autoantibody status or prednisone use) are likely to have low disease activity or remission in the third trimester. Also, women with higher disease activity who are not taking prednisone and who express no autoantibodies still have a fair chance of low disease activity in the last trimester.

INTRODUCTION

Pregnancy is the only natural situation that results in spontaneous improvement of rheumatoid arthritis (RA)[1, 2]. The most recent prospective studies have shown that RA improves during pregnancy in 40-70% of the patients[1, 3-5]. However, only 16-27% of patients achieve remission, and approximately 50% still have active disease in the third trimester[1, 4]. The treatment of pregnant patients with RA is especially challenging. First, achieving and maintaining low disease activity throughout pregnancy is of interest for the welfare of the mother. Second, since several studies have shown that active maternal disease during pregnancy is negatively associated with pregnancy outcome, maintaining low disease activity is even more important[6, 7].

Unfortunately not all antirheumatic drugs can be continued during pregnancy. Currently, mainly prednisone, sulfasalazine, hydroxychloroquine, and increasingly tumor necrosis factor (TNF) inhibitors[8, 9] are prescribed to pregnant RA patients. Furthermore, the use of a minimal amount of medication during pregnancy is preferred, to prevent drug-related side effects for mother and child. Therefore, and in view of the known improvement of RA during pregnancy, many physicians try to taper or to stop medication when a woman conceives. TNF inhibitors are often discontinued during the first weeks of pregnancy, since most safety data mainly concern first trimester exposure[8, 10]. In addition, TNF inhibitors with high affinity for the fetal fragment crystallizable (Fc)-receptor are actively transported to the fetus during the second and third trimester of pregnancy, which may result in fetal cord/serum levels equal to or higher than maternal levels[9]. Expert opinion therefore advises to preferably discontinuing these agents before week 20 of gestation[11].

Moreover, an attempt is often made to stop or to taper the more traditional disease-modifying antirheumatic drugs (DMARDs) during pregnancy. The rationale for doing so is that some side effects are mainly related to exposure later during pregnancy, e.g. prednisone has been associated with shorter gestational age[6], gestational hypertension, gestational diabetes, and premature rupture of the membranes[12], side effects that are most prominent during third-trimester exposure. Since lowering medication during pregnancy increases the risk of a flare of disease activity[13, 14], it would be useful to identify those female RA patients in whom medication can be tapered safely in the first trimester of pregnancy.

The aim of the current study was to determine which combination of clinical factors at the beginning of pregnancy was associated with low disease activity (Disease Activity Score in 28 joints[DAS28] using the C-Reactive Protein [CRP] level < 3.2) and remission (DAS28-CRP(3) < 2.6) in the third trimester. There is evidence, from univariate analyses, that the presence of autoantibodies[5], the initial DAS28-CRP(3)[4], and the use of prednisone[15] are associated with RA disease activity during pregnancy. However, these factors have not yet been analyzed together in a multivariate model and were never studied in association with

other relevant clinical factors. In the current study, we performed multivariate analyses using these known factors and possible other factors, e.g., sulfasalazine use, parity of the mother, methotrexate (MTX) use in the past, the presence of erosions, and the duration of RA.

PATIENTS AND METHODS

Study population

This study is embedded in the Pregnancy-induced Amelioration of Rheumatoid Arthritis (PARA) study, a nationwide prospective cohort study from The Netherlands, conducted to gain insight into the influence of pregnancy on the RA disease activity[4]. Patients were eligible for inclusion if they fulfilled the American College of Rheumatology 1987 revised criteria for RA[16] and had a good understanding of the Dutch language. From 2002 to 2008, 475 female RA patients who had a wish to conceive or were already pregnant (in their first trimester) were recruited by their rheumatologist, and from these, 369 were enrolled in the PARA-study. During the study period, 205 women conceived at least once.

For the current analysis on identifying associated factors with RA disease activity during pregnancy, patients with a miscarriage (within 12 weeks) and patients with a missing DAS28-CRP(3) in the first or third trimester were excluded. After these exclusions, data on 190 pregnancies from 168 women remained available for the current analysis. In 110 pregnancies, a preconception DAS28-CRP(3) was available.

Data collection

In the PARA-study, data on disease activity and medication use were collected before conception if possible, each trimester during pregnancy (8-12 weeks , 18-22 weeks, and 28-32 weeks) and 3 times postpartum (6 , 12 , and 26 weeks). The PARA-study has been described in detail previously[4]. Disease activity was measured with the DAS28-CRP(3) based on the number of swollen joints, the number of tender joints, and the CRP level, without consideration of the visual analogue scale (VAS) of global health[17,18]. Improvement in disease activity was calculated as the difference between first and third trimester DAS28-CRP(3).

Patient characteristics (duration of RA, erosion status, presence of Rheumatoid Factor[RF], and the presence of anti-citrullinated protein antibody[ACPA]) were obtained from the patient's rheumatologist. RF and ACPA were also determined at study inclusion. A patient was considered to be positive for RF or ACPA when they were positive either at inclusion or in the past[4].

Statistical analysis

For all subjects, descriptive statistics were calculated as numbers, percentages, means, medians, standard deviation (SD) scores, and interquartile ranges (IQRs). The mean DAS28-CRP(3) and the mean difference between DAS28-CRP(3) from first to third trimester and from preconception (if available) to third trimester was calculated. Paired t-tests were used to test the differences in disease activity scores.

Two multivariate logistic regression models were built for the analyses of the dichotomized DAS28-CRP(3) in the third trimester (DAS28-CRP(3) ≥ or < 2.6 and DAS28-CRP(3) ≥ or < 3.2) by step-wise forward selection of covariates with a P value less than 0.20 in the univariate logistic regression analyses. After that, the full models were reduced by eliminating the covariates with a P value greater than or equal to 0.20 in the multivariate regression analyses (if present) to create the final models.

The following independent covariates were analyzed in these models: the DAS28-CRP(3) in the first trimester, prednisone use in the first trimester, sulfasalazine use in the first trimester, parity of the mother, MTX use in the past, autoantibody status (either one or both RF and ACPA positive versus both negative), the presence of erosions, and the duration of RA. The past use of MTX was included in our model as a marker for more severe RA, since in The Netherlands at the time of inclusion for this study, MTX was mainly prescribed to patients with more severe disease. The interaction terms between DAS28-CRP(3) in the first trimester, prednisone use in the first trimester and the presence of autoantibodies were also analyzed in the final regression models. A stratified analysis was performed if interaction terms were considered significant (P < 0.20). Correlations between the covariates were calculated with Pearson's and Spearman's rank tests.

All of the above mentioned analyses were repeated in patients with a preconception DAS28-CRP(3) available. Instead of the DAS28-CRP(3) in the first trimester and prednisone and sulfasalazine use in the first trimester, the preconception DAS28-CRP(3) and preconception use of prednisone and sulfasalazine were used as independent covariates.

P values less than or equal to 0.05 were considered statistically significant, except for interaction terms, where a P value less than 0.20 was considered significant. All statistical analysis were performed using STATA software version 14.1 for Windows.

Ethics

This study is in compliance with the Helsinki Declaration, and the Medical Ethics Committee at the Erasmus Medical Center Rotterdam, The Netherlands, approved the PARA-study.

RESULTS

Participants

In total, data on 190 pregnancies from 168 women were used for the current analysis. Descriptive statistics of the study population are shown in table 1. The mean maternal age in the first trimester was 32.2 years and the median duration of RA was 4.8 years. In total, 133 patients (70.0%) were RF positive, and 116 patients (61.1%) were ACPA positive. Of these patients, 143 (75.3%) were classified as either RF or ACPA positive, or positive for both, and 47 (24.7%) as negative for both. Erosions were present in 118 patients (62.1%).

RA disease activity during pregnancy

The mean ± SD DAS28-CRP(3) changed during pregnancy from 3.6 ± 1.2 in the first trimester to 3.3 ± 1.2 in the third trimester (P < 0.001) to 3.5 ± 1.1 at 12 weeks postpartum (P = 0.01), and finally 3.4 at 26 weeks postpartum (P = 0.03). In the third trimester, 50.0% of the patients had low disease activity (DAS28-CRP(3) < 3.2) compared to 40.5% in the first trimester (P = 0.02). The total number of patients in remission (DAS28-CRP(3) < 2.6) increased from 43 (22.6%) in the first trimester to 58 (30.5%) in the third trimester (P = 0.03).

In 110 pregnancies (57.9%) there was a preconception DAS28-CRP(3) available. In this subgroup, similar results for disease activity (but in this case from preconception to third trimester) as in the whole group were obtained.

Medication use during pregnancy

In this study, the only antirheumatic medications prescribed during pregnancy were prednisone, sulfasalazine and hydroxychloroquine. Medication use remained overall similar throughout pregnancy. Prednisone, either with or without sulfasalazine and hydroxychloroquine, was used by 80 patients (42.1%) during at least 1 trimester of pregnancy. Seventy patients (36.8%) used prednisone in the first trimester, and 58 (30.5%) continued prednisone throughout the whole pregnancy. The median dose of prednisone was 7.5 mg/day (IQR 5.0 - 10) in every trimester during pregnancy.

Sulfasalazine, either with or without prednisone and hydroxychloroquine, was used by 62 patients (32.6%) during at least one trimester of pregnancy. A total of 57 patients (30.0%) used sulfasalazine in the first trimester, and 51 (26.8%) continued sulfasalazine throughout the entire pregnancy. The median dose of sulfasalazine remained 2,000 mg/day (IQR 1,500 – 2,000) in every trimester during pregnancy.

Hydroxychloroquine, either with or without prednisone and sulfasalazine, was used by 6 patients (3.2%) during at least 1 trimester of pregnancy. Five patients (2.6%) used hydroxychloroquine in the first trimester, and 4 (2.1%) continued hydroxychloroquine

Table 1. Descriptive statistics of study population. Values are number (%) unless indicated otherwise.

Baseline characteristics (n = 190)	
Age of mother, 1st trimester, mean ± SD years	32.2 ± 3.7
Smoking during pregnancy	15 (7.9)
Duration of 1st trimester RA, median (IQR) years	4.8 (2.1 – 10.0)
RA-associated autoantibodies	
RF positive	133 (70.0)
ACPA positive	116 (61.1)
Either RF or ACPA, or both positive	143 (75.3)
Presence of erosions	118 (62.1)
Parity	
Nulliparous	101 (53.2)
Multiparous	89 (46.8)
DAS28-CRP(3) 1st trimester, mean ± SD	3.6 ± 1.2
1st trimester DAS28-CRP(3)*:	
< 2.6	43 (22.6)
≥ 2.6 to < 3.2	34 (17.9)
≥ 3.2 to ≤ 5.1	95 (50.0)
> 5.1	18 (9.5)
Methotrexate use in the past	116 (61.1)
Biologic agent use in the past	33 (17.4)
Medication use during 1st trimester	
Prednisone only	45 (23.7)
Sulfasalazine only	31 (16.3)
Both prednisone and sulfasalazine	24 (12.6)
Hydroxychloroquine (either alone or in combination)	5 (2.6)
No medication during 1st trimester	85 (44.7)

RA= rheumatoid arthritis; SD= standard deviation; IQR= interquartile range; RF= rheumatoid factor; ACPA= anti-citrullinated protein antibody; DAS28-CRP(3) = Disease Activity Score in 28 joints using C-reactive protein levels. Disease activity groups are defined according to the European League Against Rheumatism criteria. * Disease activity groups are defined according to the European League Against Rheumatism criteria.

throughout the entire pregnancy. The median dose of hydroxychloroquine was 200 mg/day (IQR 200 – 400) in the first and second trimester, and 300 mg/day (IQR 200-450) in the third trimester of pregnancy.

In the first trimester, 85 patients (44.7%) did not use antirheumatic medication. From these, 74 patients (39.0%) did not use medication throughout the whole pregnancy. Of the 11 patients who started using antirheumatic medication after the first trimester, 6 started using prednisone and 5 started using sulfasalazine. Five of the patients on prednisone started using it in the second trimester. The mean DAS28-CRP(3) of the patients using prednisone was 4.8 in the first and 4.6 in the third trimester. All patients who received prednisone had a DAS28-CRP(3) in the first, second, and third trimester above 3.2. In total, 116 patients (61.1%) used MTX and 33 (17.4%) used biological agents in the past. In all patients, MTX and biological agents were discontinued prior to conception.

Factors associated with low disease activity and remission in the third trimester

For clinical purposes the DAS28-CRP(3) in the third trimester was dichotomized for easier interpretation. In addition, the DAS28-CRP(3) in the first trimester was dichotomized to < 3.2 or ≥ 3.2. In the multivariate model of low disease activity (DAS28-CRP(3) < 3.2) in the third trimester, moderate-to-high disease activity (DAS28-CRP(3) ≥ 3.2) in the first trimester, the presence of autoantibodies, and the use of prednisone during the first trimester were negatively associated with low disease activity in the third trimester (P < 0.001, 0.033, and 0.024, respectively) (Table 2). In the final model, the interaction term between the DAS28-CRP(3) in the first trimester and prednisone use in the first trimester was significant (P = 0.094).

In the multivariate logistic regression analysis of remission (DAS28-CRP(3) < 2.6) in the third trimester, only moderate-to-high disease activity (DAS28-CRP(3) ≥ 3.2) in the first trimester and the presence of autoantibodies were negatively associated with remission (DAS28-CRP(3) < 2.6) in the third trimester (P < 0.001 and P < 0.005, respectively) (Table 2). Prednisone did not reach statistical significance (P = 0.089), but there was a trend for a negative association. In the final model, the interaction term between the DAS28-CRP(3) in the first trimester and the presence of autoantibodies was significant (P = 0.033).
In the subgroup analyses of patients for whom a preconception DAS28-CRP(3) was available (n = 110), similar results as in the whole group were obtained with the preconception variables (data not shown).

Since 2 of the interaction terms were positive in these final models, subgroups based on the DAS28-CRP(3) (< 3.2 or ≥ 3.2) in the first trimester were created to perform a stratified analysis (Table 3). In patients with a low initial DAS28-CRP(3) (< 3.2) , neither prednisone use in the first trimester nor the presence of autoantibodies were associated with low disease activity or remission in the third trimester. In patients with a DAS28-CRP(3) ≥ 3.2 in the first trimester, prednisone use in the first trimester was negatively associated with low disease activity, and the absence of autoantibodies was associated with remission in the third trimester in the multivariate models (P = 0.007 and P = 0.001, respectively) (Table 3).

Based on the results of the stratified analysis, patients were divided into groups to get more insight in the chance of low disease activity and remission in the third trimester (Table 4). Since prednisone use in the first trimester was only associated with low disease activity and the presence of autoantibodies was only associated with remission in patients with an initial DAS28-CRP(3) ≥ 3.2, only these subgroups were created.

In total, 95 patients achieved low disease activity (DAS28-CRP(3) < 3.2) in the third trimester (Table 4). From the 77 patients with a DAS28-CRP(3) < 3.2 in the first trimester, the majority (74.0%) had low disease activity in the third trimester as well. From the patients with an initial DAS28-CRP(3) ≥ 3.2, the chance for low disease activity was the highest in the subgroup which did not use prednisone in the first trimester (44.8%).

In total, 58 patients achieved in remission (DAS28-CRP(3) < 2.6) in the third trimester (Table 4). From the 77 patients with a DAS28-CRP(3) < 3.2 in the first trimester, 45 (58.4%) were in remission in the third trimester. From the 91 patients who had a first trimester DAS28-CRP(3) ≥ 3.2 and autoantibodies present, only 5 (5.5%) achieved remission. In total 8 of 22 patients (36.4%) of the patients with an initial DAS28-CRP(3) ≥ 3.2 but without autoantibodies achieved remission in the third trimester.

Table 2. First trimester factors associated with RA disease activity: multivariate logistic regression models for DAS28-CRP(3) ≥ or < 2.6 and ≥ or < 3.2 in the third trimester (n = 190)

	Low disease activity (DAS28-CRP(3) < 3.2)#				Remission (DAS28-CRP(3) < 2.6)#			
	Univariate		Multivariate$		Univariate		Multivariate$	
	OR	P	OR	P	OR	P	OR	P
DAS28-CRP(3) 1st trimester ≥ 3.2	0.178*	<0.001*	0.193*	<0.001*	0.092*	<0.001*	0.095*	<0.001*
Sulfasalazine use 1st trimester	1.162	0.635	-	-	1.351	0.372	-	-
Prednisone use 1st trimester	0.437*	0.007*	0.463*	0.024*	0.488*	0.039*	0.494	0.089
Parity ≥ 1	1.043	0.884	-	-	1.326	0.372	-	-
MTX use ever	0.766	0.372	-	-	0.779	0.437	-	-
Autoantibody status positive	0.369*	0.005*	0.442*	0.033*	0.299*	0.001*	0.316*	0.005*
Presence of erosions	0.638*	0.136*	-	-	0.899	0.740	-	-
Duration of RA, years	1.008	0.712	-	-	1.002	0.934	-	-
Interaction terms								
DAS28-CRP(3) 1st trimester x prednisone use 1st trimester	-	-	0.289*	0.094*	-	-	-	-
DAS28-CRP(3) 1st trimester x autoantibody status positive	-	-	1.303	0.743	-	-	0.172*	0.033*
Prednisone use 1st trimester x autoantibody status positive	-	-	0.674	0.631	-	-	-	-

RA = rheumatoid arthritis; DAS28-CRP(3) = Disease Activity Score in 28 joints using C-reactive protein levels; OR = Odds Ratio, MTX= methotrexate.
# Based on logistic regression
$ Multivariate model after forward selection (limit for inclusion: P < 0.20) and backward selection (limit for exclusion: P ≥ 0.20)
* Statistically significant

Table 3. Subgroup analysis in patients with DAS28-CRP(3) in the first trimester < 3.2 or ≥ 3.2 with factors associated with RA disease activity in the third trimester, using multivariate logistic regression models. *

DAS28-CRP(3) from 1st to 3rd trimester	Prednisone use 1st trimester				Positive autoantibody status			
	Uni variate	P	Multi variate	P	Uni variate	P	Multi variate	P
< 3.2 (n = 77) to low disease activity (n=57)# OR	1.077	0.896	0.969	0.957	0.429	0.174	0.427	0.175
<3.2 (n=77) to remission (n=45)$ OR	0.606	0.314	0.570	0.266	0.709	0.493	0.654	0.407
≥ 3.2 (n=113) to low disease activity (n=38)# OR	0.260*	0.003*	0.282*	0.007*	0.422	0.075	0.523	0.198
≥ 3.2 (n=113) to remission (n=13)$ OR	0.398	0.181	0.580	0.460	0.102*	<0.001*	0.112*	0.001*

% In addition, all other covariates, which were not significant in the multivariate logistic regression models in Table 2, were tested in these subgroups. However, these covariates were all not significant in these analyses as well (data not shown). DAS28-CRP(3) = Disease Activity Score in 28 joints using C-reactive protein levels; RA = rheumatoid arthritis; OR = odds ratio.
# Low disease activity was DAS28-CRP(3) < 3.2 in the third trimester
$ Remission was DAS28-CRP(3) < 2.6 in the third trimester.
* Statistically significant

Table 4. DAS28-CRP(3) <3.2 or ≥3.2 in the first trimester: subgroups based on stratified multivariate logistic regression models for low disease activity and remission in the third trimester%.

	1st trimester prednisone			1st trimester autoantibodies		
	< 3.2 (n = 77)	≥ 3.2, yes (n = 46)	≥ 3.2, no (n = 67)	< 3.2 (n = 77)	≥ 3.2, positive (n = 91)	≥ 3.2, negative (n = 22)
3rd trimester						
- Low disease activity	57 (74.0)	8 (17.4)	30 (44.8)	-	-	-
< 3.2	-	-	-			
- Remission < 2.6				45 (58.4)	5 (5.5)	8 (36.4)

% Values are number (%). DAS28-CRP(3) = Disease Activity Score in 28 joints using C-reactive protein levels.

DISCUSSION

Almost all studies conducted so far on the subject of pregnancy in patients with RA have only focused on the occurrence of improvement rather than determining patient characteristics associated with disease activity. In this prospective study we showed that a low initial DAS28-CRP(3), and the absence of prednisone use and the absence of autoantibodies were associated with low RA disease activity in the third trimester. Other factors we thought to be associated with the disease course that were analyzed in this study (sulfasalazine use in the first trimester, parity of the mother, MTX use in the past, the presence of erosions, and the duration of RA) did not reach statistical significance. Of course there might be other, maybe genetic factors of mother and child that influence the improvement of RA during pregnancy[3, 19, 20]. However, this would not help us in the daily practice, since we do not have the genetic profile of every patient and her offspring. Additionally, research has recently shown that the disease course in a first pregnancy is not predictive for a subsequent pregnancy[21]. We considered the sex of the child as an influential factor, but this factor also did not reach statistical significance (data not shown). On the other hand, the sex is not known until approximately midgestation, and therefore is not useful in this context.

The mean decrease in DAS28-CRP(3) of 0.3 that we found in our study is low, but the reason for this decrease is probably, as previously reported by de Man et al.[4], due to the fact that a considerable number of patients already had low disease activity (40.5%) or were in remission (22.6%) in the first trimester. The decrease in DAS28-CRP(3) was the highest in patients with higher disease activity in the first trimester[4], but these patients are still less likely, as shown in this study, to achieve low disease activity or remission.

It could be that prednisone was a proxy for disease activity since the mean DAS28-CRP(3) was the highest in patients who used prednisone during the first trimester (3.9 vs. 3.4). However, of the 18 patients who had high disease activity in the first trimester (DAS28-CRP(3) > 5.1) 10 used prednisone and 8 did not use prednisone in the first trimester. From the 10 patients who used prednisone, in total 8 patients still had high disease activity in the third trimester. From the 8 patients with high disease activity who did not use prednisone in the first trimester, 4 still had high disease activity in the third trimester. Since these numbers are low, we cannot draw any firm conclusions. However, although speculative, we cannot rule out the idea that prednisone itself may inhibit mechanisms that lead to improvement of RA during pregnancy.

Many patients and physicians alike expect to be able to taper medication at the beginning of pregnancy in RA patients, since they rely on the spontaneous improvement of RA during pregnancy. Based on the results of our study, one may consider tapering medication in those patients who are already in remission or have low disease activity at the beginning of pregnancy, and maybe even in those with higher disease activity but

not expressing autoantibodies and not taking prednisone. For those patients that still have moderate-to-high disease activity and are taking prednisone or express autoantibodies, we advise to continue medication, if possible, throughout pregnancy. Sound evidence-based recommendations on tapering medication can only be given based upon a controlled trial in which medication is tapered in a randomized manner. Given the restrictions of pregnancy, such a trial is very unlikely to be conducted. If such a trial does take place, the patient characteristics identified in this study could be used for patient stratification.

Finally, our study has some limitations. First, none of the patients within this cohort used biological agents during pregnancy. It would be interesting to replicate the results of our current study in a cohort with patients who used biological agents during at least the first trimester of pregnancy. Second, in 42% of the patients there was no preconception DAS28-CRP(3) available. Nevertheless, the regression analysis in the subgroup of 110 patients with a preconception DAS28-CRP(3) reached comparable statistical significant results to the whole group. Furthermore, there was no significant difference between preconception DAS28-CRP(3) and first trimester DAS28-CRP(3) in these patients (P = 0.43). Third, since rheumatologists in this study were free to change medication and dosages, lowering or discontinuation of prednisone after the first trimester could cause an increased DAS28-CRP(3) in the third trimester, thereby resulting in an association between prednisone use during first trimester and more active disease during third trimester. However, our analysis showed that this was not the case in our patient group. In total, 12 patients who used prednisone in the first trimester did not use prednisone in the third trimester. From these, only 1 patient had low disease activity (DAS28-CRP(3) < 3.2) in the first trimester and ended with a higher DAS28-CRP(3) in the third trimester (DAS28-CRP(3) = 4.48). Furthermore, in total 14 patients used a lower prednisone dose in the third trimester compared to the first trimester. From these, only 2 had low disease activity (DAS28-CRP(3) < 3.2) in the first trimester and ended with a higher DAS28-CRP(3) in the third trimester (DAS28-CRP(3) 3.46 and 3.70, respectively). These findings are not likely to have induced bias in the analyses, and therefore we assume that the negative association between prednisone use in the first trimester and the DAS28-CRP(3) in the third trimester is plausible. Fourth, dichotomizing the third trimester DAS28-CRP(3) to ≥ or < 2.6 and ≥ or < 3.2 resulted in slight loss of power in the association between prednisone use and remission, but there was a trend for significance (P = 0.08). Finally, for the current study we used all 190 pregnancies from 168 women in order to enlarge the power. To exclude possible selection bias in the obtained results, we performed a subgroup analysis, including only the first participation of the patients (n = 168), and found similar results in all regression analyses (data not shown).

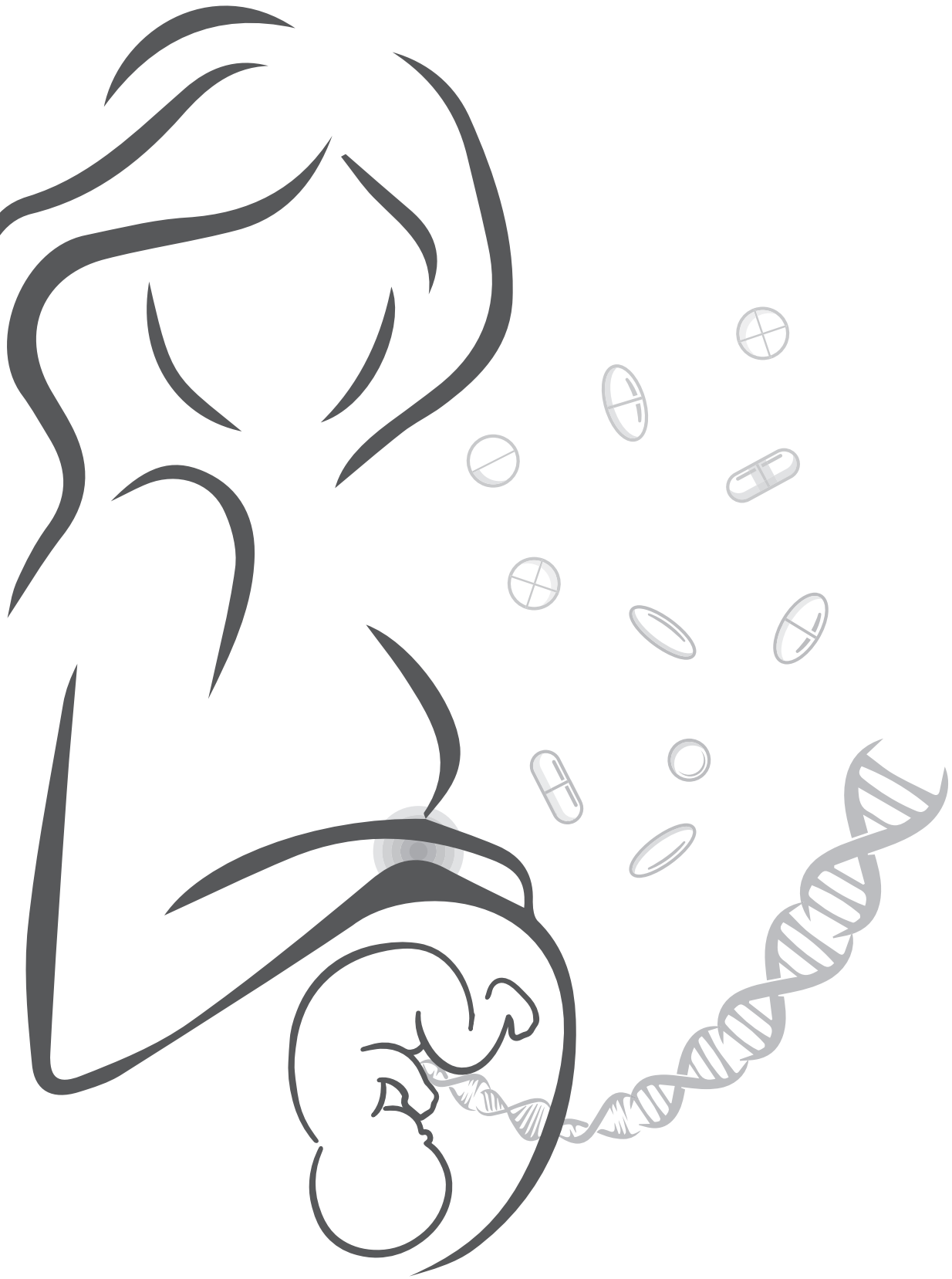
In conclusion, our study shows that a low initial DAS28-CRP(3) (preconception or first trimester), the absence of autoantibodies and the absence of prednisone use at preconception or in the first trimester are associated with low disease activity in the

third trimester. These findings create more insight into the phenomenon of spontaneous improvement of RA during pregnancy and can be helpful in the daily care for our pregnant patients with RA.

REFERENCES

1. Barrett JH, Brennan P, Fiddler M, Silman AJ. Does rheumatoid arthritis remit during pregnancy and relapse postpartum? Results from a nationwide study in the United Kingdom performed prospectively from late pregnancy. Arthritis Rheum 1999;42:1219-27.
2. Ostensen M, Husby G. A prospective clinical study of the effect of pregnancy on rheumatoid arthritis and ankylosing spondylitis. Arthritis Rheum 1983;26:1155-9.
3. Nelson JL, Hughes KA, Smith AG, Nisperos BB, Branchaud AM, Hansen JA. Maternal-fetal disparity in HLA class II alloantigens and the pregnancy-induced amelioration of rheumatoid arthritis. N Engl J Med 1993;329:466-71.
4. de Man YA, Dolhain RJ, van de Geijn FE, Willemsen SP, Hazes JM. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum 2008;59:1241-8.
5. de Man YA, Bakker-Jonges LE, Goorbergh CM, Tillemans SP, Hooijkaas H, Hazes JM, et al. Women with rheumatoid arthritis negative for anti-cyclic citrullinated peptide and rheumatoid factor are more likely to improve during pregnancy, whereas in autoantibody-positive women autoantibody levels are not influenced by pregnancy. Ann Rheum Dis 2010;69:420-3.
6. de Man YA, Hazes JM, van der Heide H, Willemsen SP, de Groot CJ, Steegers EA, et al. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: results of a national prospective study. Arthritis Rheum 2009;60:3196-206.
7. de Steenwinkel FD, Hokken-Koelega AC, de Ridder MA, Hazes JM, Dolhain RJ. Rheumatoid arthritis during pregnancy and postnatal catch-up growth in the offspring. Arthritis Rheumatol 2014;66:1705-11.
8. Hyrich KL, Verstappen SM. Biologic therapies and pregnancy: the story so far. Rheumatology (Oxford) 2014;53:1377-85.
9. Mahadevan U, Wolf DC, Dubinsky M, Cortot A, Lee SD, Siegel CA, et al. Placental transfer of anti-tumor necrosis factor agents in pregnant patients with inflammatory bowel disease. Clin Gastroenterol Hepatol 2013;11:286-92; quiz e24.
10. Ince-Aşkan H, Dolhain RJ. Pregnancy and rheumatoid arthritis. Best Pract Res Clin Rheumatol 2015;29:580-96.
11. Gotestam Skorpen C, Hoeltzenbein M, Tincani A, Fischer-Betz R, Elefant E, Chambers C, et al. The EULAR points to consider for use of antirheumatic drugs before pregnancy, and during pregnancy and lactation. Ann Rheum Dis 2016;75:795-810.
12. Bermas BL. Non-steroidal anti inflammatory drugs, glucocorticoids and disease modifying anti-rheumatic drugs for the management of rheumatoid arthritis before and during pregnancy. Curr Opin Rheumatol 2014;26:334-40.
13. Strangfeld A, Pattloch D, Spilka M, Manger B, Krummel-Lorenz B, Gräßler A, et al. OP0017 Pregnancies in patients with rheumatoid arthritis: treatment decisions, course of the disease, and pregnancy outcomes. Ann Rheum Dis 2015;74:70-1.
14. Fischer-Betz R, Sander O, Specker C, Brinks R, Schneider M. SAT0169 High risk of flares during pregnancy in women with rheumatoid arthritis who discontinue treatment with TNF-inhibitors at conception. Ann Rheum Dis 2015;74:715.
15. Quax RA, de Man YA, Koper JW, van Rossum EF, Willemsen SP, Lamberts SW, et al. Glucocorticoid receptor gene polymorphisms and disease activity during pregnancy and the postpartum period in rheumatoid arthritis. Arthritis Res Ther 2012;14:R183.
16. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
17. de Man YA, Dolhain RJ, Hazes JM. Disease activity or remission of rheumatoid arthritis before, during and following pregnancy. Curr Opin Rheumatol 2014;26:329-33.
18. de Man YA, Hazes JM, van de Geijn FE, Krommenhoek C, Dolhain RJ. Measuring disease activity and functionality during pregnancy in patients with rheumatoid arthritis. Arthritis Rheum 2007;57:716-22.
19. van der Horst-Bruinsma IE, de Vries RR, de Buck PD, van Schendel PW, Breedveld FC, Schreuder GM, et al. Influence of HLA-class II incompatibility between mother and fetus on the development and course of rheumatoid arthritis of the mother. Ann Rheum Dis 1998;57:286-90.

20. Zrour SH, Boumiza R, Sakly N, Mannai R, Korbaa W, Younes M, et al. The impact of pregnancy on rheumatoid arthritis outcome: the role of maternofetal HLA class II disparity. Joint Bone Spine 2010;77:36-40.
21. Ince-Aşkan H, Hazes JM, Dolhain RJ. Is Disease Activity in Rheumatoid Arthritis during Pregnancy and after Delivery Predictive for Disease Activity in a Subsequent Pregnancy? J Rheumatol 2016;43:22-5.



Altered DNA methylation in children born to mothers with rheumatoid arthritis during pregnancy

Hilal Ince-Aşkan
Pooja M. Mandaviya
Liesbeth Duijts
Janine F. Felix
Joyce B. J. van Meurs
Johanna M.W. Hazes
Radboud J.E.M. Dolhain

Submitted

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ABSTRACT

Objectives. Adverse exposures during early life are associated with later-life health. DNA methylation may be an underlying mechanism. The main objective of this study was to determine whether the DNA methylation profile of children born to mothers with rheumatoid arthritis (RA) is different from that of children born to mothers from the general population. In addition, we aimed to determine whether any differences in methylation are associated with maternal RA disease activity or medication use during pregnancy.

Methods. For this study, genome-wide DNA methylation was measured at CpG sites, using the Infinium Illumina Human-Methylation 450k BeadChip in 80 blood samples from children (mean age=6.8 years), born to mothers with RA. As controls, blood samples from 354 children (mean age=6.0 years) from the population-based Generation R Study were used. Linear mixed models were performed to investigate differential methylation between the groups, corrected for relevant confounders.

Results. A total of 147 CpGs were differentially methylated between blood samples of children born to mothers with RA and the control blood samples. The 5 most significantly associated CpGs were: cg06642177, cg08867893, cg06778273, cg07786668, and cg20116574. The differences in methylation were not associated with maternal RA disease activity or medication use during pregnancy

Conclusions. DNA methylation at 147 CpG sites differed between children born to mothers with RA and children born to mothers from the general population. It remains unknown whether the identified associations are causal and if so, whether they are caused by the disease or treatment. More research, including the replication of these results, is necessary in order to determine any relevance of these findings for the later-life health of children born to mothers with RA.

Adverse exposures in early life are associated with later-life health, which is referred to as the Developmental Origins of Health and Disease (DOHaD) hypothesis[1-5]. In the Dutch Hunger Winter study, exposure to famine during pregnancy has been shown to be associated with glucose intolerance, obesity, and cardiovascular and metabolic disease in adulthood[6-9].

The exact mechanisms underlying these associations are not completely understood. Epigenetic processes are thought to be one of the mechanisms underlying the associations of early-life exposures and later life health outcomes[10, 11]. Epigenetic changes are alterations to the DNA that do not affect the base pair order, but may influence gene expression. DNA methylation is the best studied and understood epigenetic modification[12, 13], and has been shown to be associated with a number of adverse *in utero* exposures. Factors that have been demonstrated to be associated with fetal DNA methylation include maternal disease[11] and malnutrition[6, 8, 14, 15], smoking[16], placental insufficiency [17], corticosteroids[18], folate depletion[19] and cytokines[20]. DNA methylation usually occurs at Cytosine-phosphate-Guanine (CpG) sites[12, 13]. The effect of hypermethylation and hypomethylation of a CpG, on the expression of genes, depends on the location of the CpGs[21, 22]. The most pronounced changes in DNA methylation occur during early pregnancy[11, 14].

During embryogenesis, there are 3 germ layers that form in the developing fetus: the ectoderm, the mesoderm, and the endoderm (e.g. buccal epithelial cells are derived from ectoderm, and blood from mesoderm). When DNA methylation is altered in early pregnancy, all the germ layers are affected[23, 24].

RA is a chronic inflammatory disorder, and may be considered as an adverse exposure during pregnancy[25]. Therefore, it is plausible that maternal RA may induce changes in fetal DNA methylation, and that it is related with the later-life health of the offspring. Interleukin (IL)-6, one of the pro-inflammatory cytokines present in patients with RA, is known to influence DNA methylation [20]. RA treatment during pregnancy includes among others sulfasalazine (SSZ) and corticosteroids such as prednisone. SSZ is a known folate antagonist and could influence DNA methylation in this respect[19]. SSZ crosses the placenta and the developing child is exposed to almost similar levels of SSZ as the mother [26]. Furthermore, corticosteroids might influence DNA methylation[18]. The active form of prednisone, prednisolone, is normally inactivated in the placenta[27-29]. However, until the gestational age of approximately 12 weeks, the phase in which a lot of DNA methylation changes happen, the placenta is not completely developed, and prednisolone diffuses passively to the fetus[30].

In the current study we investigated whether the DNA methylation profile of children born to mothers with RA was different from that of children born to mothers from the general population. Furthermore, we investigated whether the any differentially methylated CpGs were associated with RA disease activity or medication use during pregnancy, or with indicators of future metabolic and cardiovascular diseases. In addition, we examined whether these CpGs were associated with the expression of genes using expression quantitative trait methylation (eQTM) analysis.

METHODS

Study population

FEPRA-study

This study is embedded in the Pregnancy induced Amelioration of Rheumatoid Arthritis (PARA) study, a nationwide prospective cohort study from the Netherlands on pregnancy and RA[31]. From May 2002 to August 2008, 369 female RA patients who fulfilled the 1987 revised criteria of the American College of Rheumatology[32] and had a wish to conceive or were already pregnant, preferably in the first trimester, were enrolled in the PARA-study[33, 34]. After participation in the PARA-study, 196 children and their parents were invited to participate in a follow-up study on fetal programming in RA, the FEPRA-study. For this study, 108 children with a mean age of 6.8 years (SD=1.3) visited the Erasmus MC in Rotterdam. There were no statistical differences in baseline characteristics between the participating and non-participating group. The main reason for non-participation was the distance to the hospital (38%), or that parents felt that the investigations were too much of a burden for their child (30%). For the current study on epigenetics, the parents were contacted once more to obtain written informed consent to perform DNA methylation analysis in stored blood samples and to provide cheek swab samples from their children. Finally, parents of 85 children gave consent to analyse the blood samples of their (European-ancestry) children. Furthermore, parents of 71 children provided cheek swabs from their children. From the parents of 23 children who did not provide informed consent, 20 (87.0%) could not be successfully contacted by phone or mail after multiple attempts.

Generation R Study

The control group consisted of children with a mean age of 6.0 years (SD=0.4), included in the Generation R Study, a population-based prospective cohort study from pregnancy onwards in Rotterdam, the Netherlands. The Generation R Study has been described in detail previously[35]. Briefly, all pregnant women living in Rotterdam with a delivery date between April 2002 and January 2006 were invited to participate, and 9,778 mothers were enrolled in the study[35]. At the age of 6 years, DNA methylation was measured in a subgroup of 493 children of European ancestry.

Data collection

FEPRA-study

In the PARA-study, RA patients were seen before pregnancy (if possible), three times during pregnancy and again three times after the birth of the child. At all time-points, data on

mother (e.g. disease activity [with the Disease Activity Score in 28 joints using C-Reactive Protein levels (DAS28-CRP[3])], medication use, and pregnancy and outcome) and child (growth parameters) were collected [34]. For the FEPRA-study, during the visit around the age of 7 years, data on blood pressure, growth and body composition including fat percentage and lean body mass of the children were measured. Also, blood, which is a mesoderm germ layer derivate, was drawn for the DNA methylation analysis [36]. Cheek swabs were collected for the analysis of DNA methylation in buccal epithelial cells, which is an ectoderm germ layer derivate.

Generation R Study

In the Generation R Study, mothers were seen three times during pregnancy. The children were followed from birth until childhood. Data collection in children and their mothers included among others, questionnaires (e.g. medical history, medication use including the use of corticosteroids during pregnancy), detailed physical examinations, and blood sampling[35].

DNA methylation analysis

Genome-wide DNA methylation analysis

Genomic DNA was extracted from whole peripheral blood samples at the lab of the internal medicine of the Erasmus MC, and from the cheek swab samples at the rheumatology lab of the Erasmus MC. Bisulfite conversion of 500ng of genomic DNA was performed using the Zymo EZ-96 DNA Methylation Kit (Zymo Research Corporation, Irvine, CA, USA) according to the manufacturer's protocol.

Genomic methylation profiling was performed using the Infinium Illumina HumanMethylation 450k BeadChip arrays (Illumina Inc., San Diego, USA) according to the manufacturer's protocol. The Illumina array measures methylation status of 485,512 CpG sites in the gene and non-gene regions across the entire human genome. To prevent possible batch-effects, blood and cheek swab samples were measured in the same run.

Quality control and normalization

The data were pre-processed using the minfi package in R version 3.4.1 (www.r-project.org). Samples with incomplete or poor bisulfite conversion, extension, hybridization, or specificity were excluded[37]. In addition, samples with sex mismatch and samples with a call rate <95% were removed. This quality control (QC) was done separately for blood samples (Generation R and FEPRA) and for cheek swab samples (FEPRA). During QC, 5 blood and 14 cheek swab samples from the FEPRA-study were excluded, resulting in 80 and 57 samples respectively. From the Generation R blood samples, 27 were excluded due to corticosteroid use or RA

disease in the mother, and 32 were excluded during QC, resulting in 441 blood samples. In addition, in order to perform complete case analyses, 87 cases with missing data from the Generation R Study were excluded leaving 354 samples to analyse. The intensity values were then quantile normalized using the CPACOR workflow[37]. Methylation at each CpG was calculated as the beta-value [beta=intensity of the methylated allele (M)/(intensity of the unmethylated allele (U) + M + 100)], containing values from 0 to 1. Blood cell composition of the samples was estimated using the Houseman method with the Reinius reference set[38, 39]. Probes with SNPs at single base extension, probes with improper binding, and CpGs on the X and Y chromosome were removed from the analysis[40]. From the initial 485,512 CpGs, 32,057 were excluded during QC leaving 453,456 CpGs for analysis.

Statistical Analysis

Statistical Models

For all subjects, descriptive statistics were calculated as numbers, percentages, means, Standard Deviations (SDs), medians, and InterQuartile Ranges(IQRs) using stata version 14.1 (https://www.stata.com/stata14/). Student t tests and chi-squared tests were used to compare the baseline characteristics of the FEPRA-study and Generation R Study. For these analyses, P-values <0.05 were considered statistically significant.

Linear mixed models were performed to analyse differential methylation between the groups, using R. The first model was created to compare the blood samples from the FEPRA-study with the blood samples of the Generation R Study to determine whether the DNA methylation profile of children born to mothers with RA was different from that of children born to mothers from the general population. This model was corrected for biological covariates (age, BMI standard deviation scores [SDS], adjusted for age and sex according to the Dutch reference values, using the Growth Analyser[version 4.0; Growth analyser BV, Rotterdam, the Netherlands, www.growthanalyser.org], sex, gestational age at delivery, maternal age, folic acid use during pregnancy, socioeconomic status [SES], maternal smoking, and white blood cell subtypes), technical covariates (technical batch effects [array ID & position on array], and 5 hidden confounders. Technical covariates were added as random effects in the models. The hidden confounders were calculated using the *CATE* package[41,42] while correcting for the group (RA vs non-RA offsprings), all biological covariates and technical covariates. This resulted in hidden confounders that were independent of all included covariates. The *BACON* package[42] was used to correct for unobserved covariates in order to reduce test-statistic bias and inflation. The genomic inflation factor (λ)[43] was calculated. After QC 453,456 CpGs remained for analysis. Therefore, a Bonferroni-adjusted P-value of 0.05/453,456=1.10*10-7 was used[44].

CpGs were annotated for nearby genes with the Genomic Regions Enrichment of

Annotations Tool[45]. Bonferroni significant CpGs from the first analysis were then further analyzed within the blood samples of the FEPRA-study to explore if RA disease activity, prednisone use or SSZ use during pregnancy would explain the differences in methylation found in the first model. After that, 2 linear mixed models were created with the significant CpGs, to identify whether these were associated with the BMI SDS or the fat percentage SDS of the child, as indicators of future metabolic disease. These models were analysed in the 80 blood samples of the FEPRA-study. CpGs with a P-value below 0.05/147=3.4*10^{-4} were considered statistically significant.

The significant CpGs found in the first analysis were also analyzed in DNA derived from buccal epithelial cells, obtained by cheek swabs from the FEPRA-study, to explore if the differentially methylated CpGs were also differentially methylated in that germ layer derivate, as a kind of validation of the results. For this analysis, CpGs with a p-value below Bonferroni significance level of 3.4*10^{-4} were considered statistically significant.

Genomic (median) methylation was also calculated from the beta values per sample in the blood samples of the FEPRA-study. This was compared with genomic methylation of the blood samples from the Generation R Study using a linear mixed model with the beta-value as dependent variable and the covariates as previously mentioned in the first model (including RA exposure) as confounders. A p-value of <0.05 was considered statistically significant in this model.

Expression quantitative trait methylation (eQTM) analysis

EQTMs are sites at which DNA methylation is known to influence the expression of one or more genes[46]. To analyse whether any of the significant CpGs were linked to the expression of nearby genes, an eQTM analysis was performed. For the these analyses the online BIOS QTL browser (https://molgenis26.target.rug.nl/downloads/biosqtlbrowser/) was used[40].

Ethics

This study is in compliance with the Helsinki Declaration. Informed consent was obtained for all participants. The Medical Ethics Committee of Erasmus MC, University Medical Center Rotterdam, the Netherlands, approved the PARA-study (MEC-214.320/2002/117), FEPRA-study (MEC-2009-441), and the Generation R Study (MEC198.782.2001.31)

RESULTS

Participants

The flow-chart of the study population is shown in figure 1. A total of 80 blood and 57 cheek swab samples from the FEPRA-study (children born to mothers with RA), and 354 blood samples from the Generation R Study (children born to mothers from the general population) remained for analysis.

Descriptive statistics of the study population are presented in table 1. Overall, children born to mothers with RA were slightly older compared with children in the Generation R Study (6.8 years [SD 1.3] versus 6.0 years [SD 0.4], p<0.001). In the FEPRA-study 36 women (45%) did not start using folic acid before or in early pregnancy, while in the Generation R Study the vast majority of the women (92.7%) started using folic acid before or in early pregnancy (p<0.001). Approximately half of the women (47.5%) from the FEPRA-study had a high SES based on education level compared with 68.4% of the women from the Generation R Study (p<0.001). One woman (1.3%) from the FEPRA-study, and 86 women (24.3%) from the Generation R Study smoked periconceptionally or at any time during pregnancy (p<0.001).

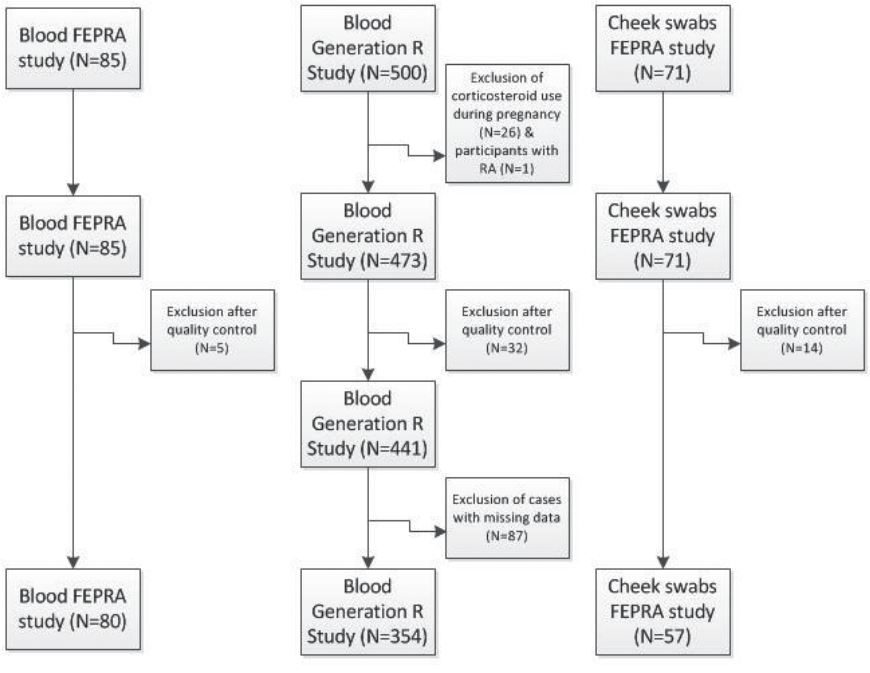


Figure 1. Flow-chart of the study population and exclusion of participants.

Table 1. Descriptive statistics of study population

	FEPRA-study (n=80)	Generation R Study (n=354)
Age child[#] (years), mean(SD)	6.8 (1.3)*	6.0 (0.4)*
BMI SDS child[#], mean(SD)	-0.14 (0.87)*	0.18 (0.74)*
Sex child		
Male, N(%)	46 (57.5)	176 (49.7)
Female, N(%)	34 (42.5)	178 (50.3)
Maternal age at delivery (years), mean(SD)	32.9 (3.9)	32.5 (4.0)
Gestational age (weeks), mean(SD)	39.5 (2.0)*	40.2 (1.5)*
Folic acid		
Start before pregnancy, N(%)	25 (31.3)*	212 (59.9)*
Start in early pregnancy, N(%)	19 (23.8)*	116 (32.8)*
No use, N(%)	36 (45.0)*	26 (7.3)*
SES based on education level		
Low, N(%)	9 (11.3)*	4 (1.1)*
Middle, N(%)	33 (41.3)*	108 (30.5)*
High, N(%)	38 (47.5)*	242 (68.4)*
Maternal smoking[$], N(%)	1 (1.3)*	86 (24.3)*
DAS28-CRP-3 3rd trimester, mean(SD)	3.3 (1.1)	-
Use of medication ≥ 1 trimester		
Only prednisone use, N(%)	17 (21.3)	-
Only sulfasalazine use, N(%)	14 (17.5)	-
Combination, N(%)	13 (16.3)	-
No medication use, N (%)	36 (45.0)	-
Fat percentage SDS, mean(SD)	0.24 (0.97)	-

BMI= Body Mass Index; SDS= Standard Deviation Score; SES= Socioeconomic Status; DAS28-CRP(3)= Disease Activity Score in 28 joints using C-reactive protein levels;
[#]At time of the blood sampling; [$]During pregnancy. *P-values <0.001

DNA methylation in the FEPRA-study as compared to the Generation R Study

In the first linear mixed model blood samples from the FEPRA-study were compared with blood samples from the Generation R Study, corrected for the covariates mentioned in the methods. In total, 147 CpGs were significantly differentially methylated between children in the FEPRA-study and children in the Generation R Study (Figure 2). The QQ-plot is shown in figure 3. The genomic inflation factor (λ) was 1.06.

In table 2 the 5 most significant CpGs, and the 5 CpGs with the largest effect sizes (within the significant CpGs) are described in detail. The complete list of significantly associated CpGs is provided as a supplemental file. A positive beta represents higher methylation levels in children born to mothers with RA. Methylation was higher in children born to mothers with RA (FEPRA-study) than in those from the general population at all 5 CpGs with the lowest P-values. Methylation at the 5 CpGs with the largest effect sizes was lower in children born to mothers with RA, with the exception of cg06656994.

The median genomic methylation level was significantly lower, albeit with a very small difference, in children born to mothers from the FEPRA-study compared with children born to mothers from the Generation R Study (effect size= -0.0003, SE=0.0002, p-value 0.038).

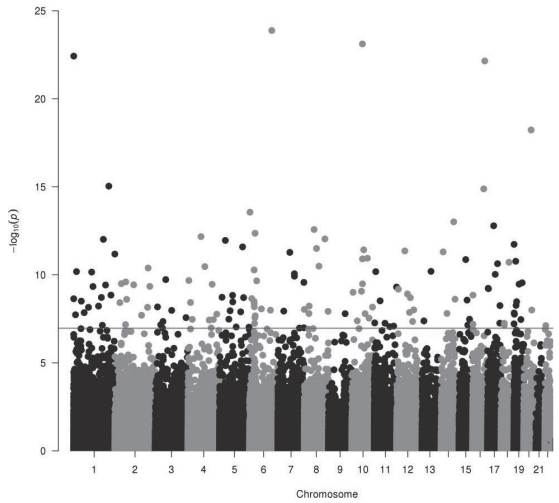


Figure 2. Results of the analysis of DNA methylation in children born to mother with RA (FEPRA-study) compared with children born to women from the general population (Generation R Study). The chromosomes are depicted on the x-axis, and the -log p-value on the y-axis. The horizontal grey line represents the Bonferroni threshold for significance of p=1.10*10-7.

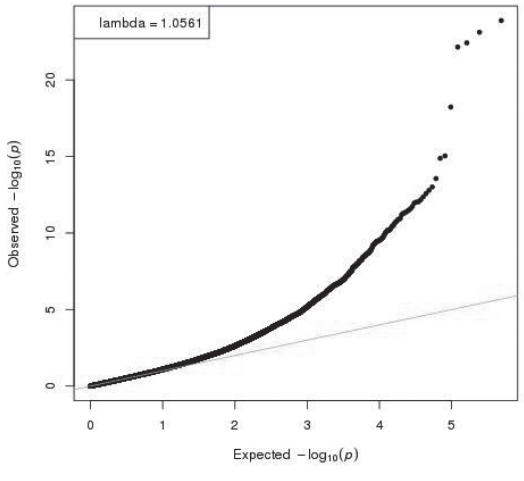


Figure 3. QQ-plot of observed versus expected p-values from the analysis of DNA methylation in children born to mother with RA (FEPRA-study) compared with children born to women from the general population (Generation R Study).

Table 2. The 5 most significant CpGs (white rows) and the 5 CpGs with the largest effect size (grey rows) from the linear mixed model: DNA methylation in blood samples from children born to mothers with RA (FEPRA-study) compared with children born to mothers from the general population (Generation R Study).

CpG	Beta[$]	SE	P-value	Nearest gene (±bp)	Chr	Bp	Location[#]
cg06642177	0.028	0.002	$1.32*10^{-24}$	*SLC2A12*(-122529)	6	134496341	-
cg08867893	0.018	0.002	$7.66*10^{-24}$	*ZNF365*(+221)	10	64134160	-
cg06778273	0.024	0.002	$3.77*10^{-23}$	*TNFRSF18*(+4995)	1	1137117	-
cg07786668	0.026	0.002	$7.11*10^{-23}$	*ZFHX3*(-10142)	16	73092391	-
cg20116574	0.019	0.002	$5.91*10^{-19}$	*NCOA5*(+435)	20	44718168	Promoter
cg16930947	-0.050	0.008	$3.22*10^{-11}$	-	8	88984447	-
cg01485645	-0.044	0.006	$1.64*10^{-13}$	*MLLT6*(+303)	17	36862199	Promoter
cg12360123	-0.043	0.008	$1.61*10^{-08}$	-	10	79984532	Enhancer
cg06656994	0.038	0.005	$9.67*10^{-13}$	*FAM163A*(+903)	1	179713176	Enhancer
cg17483482	-0.037	0.006	$4.62*10^{-10}$	-	1	117152162	-

bp=base pair; Chr= chromosome; SE= standard error
[$] Beta represents the difference in DNA methylation at the given CpG site in children born to mothers with RA (FEPRA-study) as compared with children born to mothers from the general population (Generation R Study)
[#] Location in promoter, enhancer or unknown (-)

Association of the differences in DNA methylation with maternal RA disease activity and medication use during pregnancy and with indicators for future metabolic disease

None of the 147 CpGs were significantly associated with disease activity (DAS28-CRP(3)) or medication (prednisone or SSZ) use. In addition, none of the CpGs were associated with BMI SDS or fat percentage SDS in the children.

Analysis in buccal epithelial cells

A total of 10 out of the 147 CpGs significantly associated with maternal RA in blood were also associated in buccal epithelial cells. From these, 4 were in the same direction as in blood, table 3. CpG cg11336323 was located in a promoter region.

Table 3. CpGs that were differentially methylated in the same direction in both blood and in buccal epithelial cells.

CpG	Beta[$]	SE	P-value	Nearest gene (±bp)	Chr	Bp	Location[#]
cg22998206	0.1029	0.022	$4.40*10^{-06}$	-	12	49239429	-
cg03654106	0.0727	0.016	$9.50*10^{-06}$	-	19	49539527	-
cg02613964	-0.058	0.014	$7.57*10^{-05}$	-	3	44690321	-
cg11336323	-0.092	0.024	$1.63*10^{-04}$	-	19	41946040	Promoter

bp=base pair; Chr= chromosome; SE= standard error
[$] Beta represents the difference in DNA methylation at the given CpG site in buccal epithelial cells from children born to mothers with RA (FEPRA-study) as compared with blood samples from children born to mothers from the general population (Generation R Study)
[#] Location in promoter, gene, enhancer or unknown (-)

Expression Quantitative Trait Methylation (eQTM) analysis

Two CpGs, cg21384971 and cg11220663, were associated with expression of the *COPZ2* and *ADD2* genes, respectively (Table 4). These two genes were also the nearest genes to those CpGs[45]. Both CpGs were hypermethylated in the children born to mothers with RA (FEPRA-study) as compared with children born to mothers from the general population (Generation R Study), and were associated with decreased expression of COPZ2 and ADD2 in the BIOS eQTM lookup browser.

Table 4. Results from the eQTMs analysis using the 147 CpGs significantly different in children born to mothers with RA (FEPRA-study).

CpG	Beta[$]	SE[$]	P-value[$]	Nearest genes	Beta GN#	SE GN#	P-value GN#	Genes GN#
cg21384971	0.029	0.004	$3.86*10^{-13}$	*COPZ2*	-0.073	0.039	$1.79*10^{-06}$	COPZ2
cg11220663	0.023	0.003	$1.68*10^{-11}$	*ADD2*	-0.121	0.039	$3.32*10^{-06}$	ADD2

SE= standard error; GN= Genenetwork
[$] The columns Beta, SE, P-value, and nearest genes represent the results from the analysis of DNA methylation in children born to mothers with RA (FEPRA-study) as compared with children born to mothers from the general population (Generation R Study)
The columns Beta GN, SE GN, P-value GN, and Genes GN represent the results from the BIOS eQTM lookup browser.
The positive beta's in column "Beta" represent hypermethylation, the negative beta's in column "Beta GN" represent decreased gene expression

DISCUSSION

This is the first study investigating the differences in DNA methylation of children born to mothers with RA compared with children born to mothers from the general population. In this unique study, all participants were followed prospectively from pregnancy onwards. Our study showed differential DNA methylation between the two groups. The differentially methylated CpG sites were not associated with disease activity (DAS28-CRP(3)) and/or medication (prednisone or SSZ) use, nor to BMI SDS and fat percentage SDS.

In total, 147 CpGs were significantly associated with maternal RA after adjustment for multiple biological and technical covariates, and hidden confounders. In addition, although the difference was relatively small, the median genomic methylation was significantly lower in children born to mothers with RA compared with children born to mothers from the general population. A decrease in genomic DNA methylation has been found in a variety of common age-related diseases[47]. Of the 5 most significant CpGs, interestingly, 2 (cg06642177 and cg07786668) have been associated with myocardial infarction[48]. The most significant CpG, cg06642177, is located on chromosome 6 near the *SLC2A12* gene[49], associated with insulin sensitivity[49, 50], heart failure and diabetes[51] in animal models. Cg07786668, located on chromosome 16, is located in the *ZFHX3* gene. *ZFHX3* has been associated in multiple human studies with atrial fibrillation[52-56], coronary heart disease[57], and obesity in a Korean population[58]. Cg20116574, was annotated to the *NCOA5*, a protein coding gene, which has been associated with diabetes mellitus type 2 in animal models[59, 60].

From the remaining 142 CpGs, 1 (cg17218495), annotated to the *SMARCA4* gene, has independently and significantly been associated with myocardial infarction[48]. The other significant CpGs from our study have not been associated with disease phenotypes.

Out of the initial 147 significant CpGs, 10 were also significantly differentially methylated in buccal epithelial cells obtained by cheek swabs. From these, 4 were in the same direction as in blood. When DNA methylation is altered in more than one germ layer derivate, it is likely that these alterations occurred in early development[23, 24]. Unfortunately, in the Generation R Study, DNA methylation in buccal epithelial cells was not available. These 4 CpGs have not been associated with disease phenotypes in humans.

Thus, some of the associated CpGs (cg06642177, cg07786668, and cg17218495) have been associated with cardiovascular disease in previous studies, while others are located in or near genes that are associated with cardiovascular or metabolic disease. Maternal RA during pregnancy, a chronic inflammatory disease, might be associated with later-life health and disease risk in the offspring.

None of the significant CpGs were associated with RA disease activity or medication use during pregnancy. However, this may have been due to a lack of power, since these analyses were performed in the 80 samples of the children born to women with RA (FEPRA-study).

The same power problem also applied to the analysis of the CpGs with indicators for future metabolic and cardiovascular disease (BMI SDS and fat percentage).

Our study has some limitations. First, although in its kind it is a large study, a study on DNA methylation including 80 subjects and 354 controls is still relatively small. A larger sample size would increase power and might result in increased distinctive capability regarding e.g. the relationship of altered methylation with RA disease activity or medication use. Despite the relative small sample size, a large number of CpGs reached Bonferroni significance. Correcting for biological and technical covariates, as well as hidden confounders, and using *BACON* resulted in a λ near 1, reflecting that there was no inflation. Second, we were not able to collect a new independent cohort of children born to mothers with RA to replicate the results. At the time our study was performed, there were no other comparable prospective studies available. Currently, European research groups are conducting new prospective cohort studies on the impact of RA on pregnancy and offspring. We encourage these research groups, possibly with international collaborations, to replicate our study.

Thus, the results of this study may support follow-up research of children born to mothers with RA. Since follow-up research cannot cover all aspects of development of these children, it is important to make rational choices what aspects have to be studied. Based upon our data, we recommend that at least indicators for future cardiovascular and metabolic disease should be considered. The effects of RA disease activity and medication use on DNA methylation should be investigated in studies with larger sample sizes. Furthermore, in the last years the use of TNF inhibitors during pregnancy in patients with RA has increased. This often results in lower RA disease activity during pregnancy. Future research should also cover the effects of the use of TNF inhibitors on the differentially methylated CpGs in children born to mothers with RA.

In conclusion, maternal RA during pregnancy is associated with differential DNA methylation in offspring. It remains unknown whether the identified associations are causal and if so, whether they are caused by the disease or treatment. Some of the differentially methylated CpGs or their nearby genes were associated with cardiovascular or metabolic disease. Maternal RA disease might have life-long consequences for the offspring. However, more research in this particular field must be undertaken in order to strengthen the relevance of our findings.

REFERENCES

1. Barouki R, Gluckman PD, Grandjean P, Hanson M, Heindel JJ. Developmental origins of non-communicable disease: implications for research and public health. Environ Health 2012;11:42.
2. Barker DJ. Fetal origins of cardiovascular disease. Ann Med 1999;31 Suppl 1:3-6.
3. Ong KK, Dunger DB. Perinatal growth failure: the road to obesity, insulin resistance and cardiovascular disease in adults. Best Pract Res Clin Endocrinol Metab 2002;16:191-207.
4. Painter RC, de Rooij SR, Bossuyt PM, Simmers TA, Osmond C, Barker DJ, et al. Early onset of coronary artery disease after prenatal exposure to the Dutch famine. Am J Clin Nutr 2006;84:322-7; quiz 466-7.
5. Stein AD, Kahn HS, Rundle A, Zybert PA, van der Pal-de Bruin K, Lumey LH. Anthropometric measures in middle age after exposure to famine during gestation: evidence from the Dutch famine. Am J Clin Nutr 2007;85:869-76.
6. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A 2008;105:17046-9.
7. Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. Early Hum Dev 2006;82:485-91.
8. Tobi EW, Slieker RC, Luijk R, Dekkers KF, Stein AD, Xu KM, et al. DNA methylation as a mediator of the association between prenatal adversity and risk factors for metabolic disease in adulthood. Sci Adv 2018;4:eaao4364.
9. Schulz LC. The Dutch Hunger Winter and the developmental origins of health and disease. Proceedings of the National Academy of Sciences of the United States of America 2010;107:16757-8.
10. Turan N, Ghalwash MF, Katari S, Coutifaris C, Obradovic Z, Sapienza C. DNA methylation differences at growth related genes correlate with birth weight: a molecular signature linked to developmental origins of adult disease? BMC Med Genomics 2012;5:10.
11. Hogg K, Price EM, Hanna CW, Robinson WP. Prenatal and perinatal environmental influences on the human fetal and placental epigenome. Clin Pharmacol Ther 2012;92:716-26.
12. Jin B, Li Y, Robertson KD. DNA methylation: superior or subordinate in the epigenetic hierarchy? Genes Cancer 2011;2:607-17.
13. Day K, Waite LL, Thalacker-Mercer A, West A, Bamman MM, Brooks JD, et al. Differential DNA methylation with age displays both common and dynamic features across human tissues that are influenced by CpG landscape. Genome Biol 2013;14:R102.
14. Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, et al. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Hum Mol Genet 2009;18:4046-53.
15. Tobi EW, Goeman JJ, Monajemi R, Gu H, Putter H, Zhang Y, et al. DNA methylation signatures link prenatal famine exposure to growth and metabolism. Nature Communications 2014;5:5592.
16. Joubert BR, Haberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, et al. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. Environ Health Perspect 2012;120:1425-31.
17. Park JH, Stoffers DA, Nicholls RD, Simmons RA. Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. J Clin Invest 2008;118:2316-24.
18. Crudo A, Petropoulos S, Moisiadis VG, Iqbal M, Kostaki A, Machnes Z, et al. Prenatal synthetic glucocorticoid treatment changes DNA methylation states in male organ systems: multigenerational effects. Endocrinology 2012;153:3269-83.
19. Glier MB, Green TJ, Devlin AM. Methyl nutrients, DNA methylation, and cardiovascular disease. Mol Nutr Food Res 2014;58:172-82.
20. Foran E, Garrity-Park MM, Mureau C, Newell J, Smyrk TC, Limburg PJ, et al. Upregulation of DNA methyltransferase-mediated gene silencing, anchorage-independent growth, and migration of colon cancer cells by interleukin-6. Mol Cancer Res 2010;8:471-81.
21. Ruemmele FM, Garnier-Lengline H. Why are genetics important for nutrition? Lessons from epigenetic research. Ann Nutr Metab 2012;60 Suppl 3:38-43.

22. Slieker RC, Bos SD, Goeman JJ, Bovee JV, Talens RP, van der Breggen R, et al. Identification and systematic annotation of tissue-specific differentially methylated regions using the Illumina 450k array. Epigenetics Chromatin 2013;6:26.
23. Seisenberger S, Andrews S, Krueger F, Arand J, Walter J, Santos F, et al. The dynamics of genome-wide DNA methylation reprogramming in mouse primordial germ cells. Mol Cell 2012;48:849-62.
24. Seisenberger S, Peat JR, Hore TA, Santos F, Dean W, Reik W. Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. Philos Trans R Soc Lond B Biol Sci 2013;368:20110330.
25. de Man YA, Hazes JM, van der Heide H, Willemsen SP, de Groot CJ, Steegers EA, et al. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: results of a national prospective study. Arthritis Rheum 2009;60:3196-206.
26. Jarnerot G, Into-Malmberg MB, Esbjorner E. Placental transfer of sulphasalazine and sulphapyridine and some of its metabolites. Scand J Gastroenterol 1981;16:693-7.
27. D'Anna-Hernandez KL, Ross RG, Natvig CL, Laudenslager ML. Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol. Physiol Behav 2011;104:348-53.
28. Benediktsson R, Calder AA, Edwards CR, Seckl JR. Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. Clin Endocrinol (Oxf) 1997;46:161-6.
29. Burton PJ, Waddell BJ. Dual function of 11beta-hydroxysteroid dehydrogenase in placenta: modulating placental glucocorticoid passage and local steroid action. Biol Reprod 1999;60:234-40.
30. de Steenwinkel FD, Hokken-Koelega AC, Hazes JM, Dolhain RJ. The influence of foetal prednisone exposure on the cortisol levels in the offspring. Clin Endocrinol (Oxf) 2014;80:804-10.
31. de Man YA, Dolhain RJ, van de Geijn FE, Willemsen SP, Hazes JM. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum 2008;59:1241-8.
32. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
33. Ince-Aşkan H, Hazes JM, Dolhain RJ. Is Disease Activity in Rheumatoid Arthritis during Pregnancy and after Delivery Predictive for Disease Activity in a Subsequent Pregnancy? J Rheumatol 2016;43:22-5.
34. Ince-Aşkan H, Hazes JM, Dolhain RJ. Identifying clinical factors associated with low disease activity and remission of rheumatoid arthritis during pregnancy. Arthritis Care Res (Hoboken) 2016;in press.
35. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol 2016;31:1243-64.
36. de Steenwinkel FDO, Dolhain R, Hazes JMW, Hokken-Koelega ACS. Does prednisone use or disease activity in pregnant women with rheumatoid arthritis influence the body composition of their offspring? Reprod Toxicol 2017;71:118-23.
37. Lehne B, Drong AW, Loh M, Zhang W, Scott WR, Tan ST, et al. A coherent approach for analysis of the Illumina HumanMethylation450 BeadChip improves data quality and performance in epigenome-wide association studies. Genome Biol 2015;16:37.
38. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics 2012;13:86.
39. Reinius LE, Acevedo N, Joerink M, Pershagen G, Dahlen SE, Greco D, et al. Differential DNA methylation in purified human blood cells: implications for cell lineage and studies on disease susceptibility. PLoS One 2012;7:e41361.
40. Bonder MJ, Luijk R, Zhernakova DV, Moed M, Deelen P, Vermaat M, et al. Disease variants alter transcription factor levels and methylation of their binding sites. Nat Genet 2017;49:131-8.
41. Wang J, Zhao Q, Hastie T, Owen A. Confounder adjustment in multiple hypothesis testing. arXiv:150804178 2015;Submitted.
42. van Iterson M, van Zwet EW, Consortium B, Heijmans BT. Controlling bias and inflation in epigenome- and transcriptome-wide association studies using the empirical null distribution. Genome Biol 2017;18:19.
43. Devlin B, Roeder K. Genomic control for association studies. Biometrics 1999;55:997-1004.
44. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310:170.
45. McLean CY, Bristor D, Hiller M, Clarke SL, Schaar BT, Lowe CB, et al. GREAT improves functional interpretation of cis-regulatory regions. Nat Biotechnol 2010;28:495-501.

46. Jones MJ, Fejes AP, Kobor MS. DNA methylation, genotype and gene expression: who is driving and who is along for the ride? Genome Biol 2013;14:126.
47. Baccarelli A, Rienstra M, Benjamin EJ. Cardiovascular Epigenetics: Basic Concepts and Results from Animal and Human Studies. Circulation Cardiovascular genetics 2010;3:567-73.
48. Nakatochi M, Ichihara S, Yamamoto K, Naruse K, Yokota S, Asano H, et al. Epigenome-wide association of myocardial infarction with DNA methylation sites at loci related to cardiovascular disease. Clinical Epigenetics 2017;9:54.
49. Matsuzaka T, Shimano H. GLUT12: a second insulin-responsive glucose transporters as an emerging target for type 2 diabetes. J Diabetes Investig 2012;3:130-1.
50. Purcell SH, Aerni-Flessner LB, Willcockson AR, Diggs-Andrews KA, Fisher SJ, Moley KH. Improved insulin sensitivity by GLUT12 overexpression in mice. Diabetes 2011;60:1478-82.
51. Jimenez-Amilburu V, Jong-Raadsen S, Bakkers J, Spaink HP, Marin-Juez R. GLUT12 deficiency during early development results in heart failure and a diabetic phenotype in zebrafish. J Endocrinol 2015;224:1-15.
52. Benjamin EJ, Rice KM, Arking DE, Pfeufer A, van Noord C, Smith AV, et al. Variants in ZFHX3 are associated with atrial fibrillation in individuals of European ancestry. Nat Genet 2009;41:879-81.
53. Li C, Wang F, Yang Y, Fu F, Xu C, Shi L, et al. Significant association of SNP rs2106261 in the ZFHX3 gene with atrial fibrillation in a Chinese Han GeneID population. Hum Genet 2011;129:239-46.
54. Liu Y, Ni B, Lin Y, Chen XG, Fang Z, Zhao L, et al. Genetic polymorphisms in ZFHX3 are associated with atrial fibrillation in a Chinese Han population. PLoS One 2014;9:e101318.
55. Kao YH, Hsu JC, Chen YC, Lin YK, Lkhagva B, Chen SA, et al. ZFHX3 knockdown increases arrhythmogenesis and dysregulates calcium homeostasis in HL-1 atrial myocytes. Int J Cardiol 2016;210:85-92.
56. Zaw KTT, Sato N, Ikeda S, Thu KS, Mieno MN, Arai T, et al. Association of ZFHX3 gene variation with atrial fibrillation, cerebral infarction, and lung thromboembolism: An autopsy study. J Cardiol 2017;70:180-4.
57. Sun S, Zhang W, Chen X, Song H. The CAA repeat polymorphism in the ZFHX3 gene is associated with risk of coronary heart disease in a Chinese population. Tohoku J Exp Med 2015;235:261-6.
58. Yang SA. Association study between ZFHX3 gene polymorphisms and obesity in Korean population. J Exerc Rehabil 2017;13:491-4.
59. Gao S, Li A, Liu F, Chen F, Williams M, Zhang C, et al. NCOA5 Haplo-insufficiency Results in Glucose Intolerance and Subsequent Hepatocellular Carcinoma. Cancer cell 2013;24:725-37.
60. Liu CY, Feng G-S. NCOA5, a molecular link between type 2 diabetes and liver cancer. Hepatobiliary Surgery and Nutrition 2014;3:106-8.

Supplemental table. The 147 significant CpGs from the linear mixed model: DNA methylation in blood samples from children born to mothers with RA(FEPRA-study) compared with children born to mothers from the general population(Generation R Study).

CpG	Nearest gene (+/- base pairs)	Chr	Base pairs	Location	Beta	Standard error	P-value
cg06642177	SLC2A12(-122529	6	134496341	-	0.027693315	0.002704662	1.32E-24
cg08867893	ZNF365(+221)	10	64134160	-	0.018410514	0.001828632	7.66E-24
cg06778273	TNFRSF18(+4995)	1	1137117	-	0.022857221	0.002306493	3.77E-23
cg07786668	ZFHX3(-10142)	16	73092391	-	0.02586352	0.002626713	7.11E-23
cg20116574	NCOA5(+435)	20	44718168	Promoter	0.018815575	0.002115599	5.91E-19
cg21160472	ATF3(+119)	1	212782112	Promoter	0.013379402	0.00166464	9.18E-16
cg09000178	CBFB(+294)	16	67063319	-	0.014919311	0.001866453	1.31E-15
cg01067849	WRNIP1(-102)	6	2765587	Promoter	0.010168425	0.001336479	2.78E-14
cg14119392	DICER1(-15866)	14	95623926	Promoter	0.009496235	0.001275776	9.81E-14
cg01485645	MLLT6(+303)	17	36862199	Promoter	-0.044105009	0.005979805	1.64E-13
cg10196289	NSMAF(-196)	8	59572185	-	0.013826063	0.001891212	2.66E-13
cg13018448	,CUTA(+600)	6	33385440	Promoter	0.035622707	0.004917127	4.34E-13
cg01402409	FRAS1(-614)	4	78978133	-	0.034187754	0.004758592	6.75E-13
cg17492326	,ANXA13(-31076)	8	124780746	Promoter	0.013530835	0.001894369	9.15E-13
cg06656994	FAM163A(+903)	1	179713176	Enhancer	0.038178019	0.0053507	9.67E-13
cg13390975	BRIX1(+95)	5	34915890	Promoter	0.012727324	0.001788634	1.11E-12
cg17667988	PTBP1(-73)	19	797342	Promoter	-0.033538136	0.004760613	1.86E-12
cg14810343	CXXC5(-127)	5	139028149	-	0.011939828	0.001706185	2.60E-12
cg17662034	RDH10(+706)	8	74207518	-	0.01366994	0.001961263	3.17E-12
cg22247188	H2AFY2(+1271)	10	71813603	-	0.020553561	0.002959855	3.81E-12
cg22998206	-	12	49239429	-	0.031783604	0.004590532	4.40E-12
cg00742472	HEATR5A(-149)	14	31889912	Promoter	-0.036649507	0.005307146	5.00E-12
cg21423973	TYW1B(+121)	7	72298667	Promoter	0.034153776	0.004952153	5.32E-12
cg24334803	ZNF672(+281)	1	249132834	Promoter	0.032219477	0.00469291	6.62E-12
cg11014794	CPEB3(+51162)	10	93999688	-	0.020288544	0.002988909	1.14E-11
cg15591678	ZNF365(+216)	10	64134155	-	0.023828478	0.003516927	1.24E-11
cg08700690	RORA(+35122)	15	60884630	Promoter	0.009395729	0.001389406	1.36E-11
cg17218495	SMARCA4(+170)	19	11071743	Promoter	0.010408213	0.001546442	1.69E-11
cg18238491	SKA1(+73)	18	47901440	-	0.006920639	0.0010313	1.94E-11
cg00476896	-	17	60215463	-	-0.029335846	0.004389098	2.33E-11
cg16930947	-	8	88984447	-	-0.049809031	0.007505862	3.22E-11
cg01458961	PPP3CA(-822)	4	102269425	-	0.022397284	0.003379311	3.41E-11
cg22704520	TYW5(+31)	2	200820451	Promoter	0.015040524	0.002278848	4.11E-11

Supplemental table. Continued.

CpG	Nearest gene (+/- base pairs)	Chr	Base pairs	Location	Beta	Standard error	P-value
cg25863503	-	6	27799295	-	0.018114832	0.002760384	5.29E-11
cg23792383	KLF5(+171)	13	73633288	Promoter	0.009986606	0.001528091	6.35E-11
cg03847293	-	1	17734508	-	-0.032462167	0.004970659	6.54E-11
cg08561325	AKIP1(+289)	11	8932965	Promoter	0.016285887	0.002494156	6.59E-11
cg12100751	HENMT1(+95)	1	109203672	Promoter	0.012782918	0.001960274	6.98E-11
cg18342832	-	7	99933797	Promoter	0.008837976	0.0013622	8.70E-11
cg21384971	COPZ2(+601)	17	46114574	-	0.029200877	0.004508859	9.40E-11
cg17607973	MEPCE(+179)	7	100027408	Promoter	0.012264221	0.001904559	1.20E-10
cg22512322	PSMD6(+47)	3	64009096	Promoter	0.010619257	0.001666666	1.87E-10
cg17255450	EVC2(-55)	4	5710372	-	0.016872706	0.002655126	2.09E-10
cg16041611	SRF(+785)	6	43139680	-	0.010561056	0.001664015	2.20E-10
cg11877270	SPRED2(+1048)	2	65658583	-	0.014862583	0.002349815	2.53E-10
cg25707994	DNAJB6(0)	7	157129685	Promoter	0.014060725	0.002226484	2.70E-10
cg03654106	-	19	49539527	-	0.026109337	0.004139079	2.83E-10
cg07479988	DHX57(-281)	2	39103277	Promoter	0.023103709	0.003673311	3.18E-10
cg21538902	ADO(+464)	10	64565003	Promoter	0.018717869	0.00297763	3.25E-10
cg19031844	ZNF536(+347411)	19	31210714	Enhancer	0.026178619	0.004168745	3.39E-10
cg06295548	-	4	146296778	-	-0.021299828	0.003393384	3.45E-10
cg06117184	CKAP2L(+70)	2	113522207	Promoter	0.008425305	0.00134451	3.69E-10
cg04910183	CDC73(-75)	1	193090988	Promoter	0.010437417	0.001666679	3.79E-10
cg10181911	RPE(-268)	2	210867059	Promoter	0.035764362	0.005736336	4.53E-10
cg17483482	-	1	117152162	-	-0.036832852	0.005910728	4.62E-10
cg02461956	NCAPD3(+509)	11	134093940	Promoter	0.01486645	0.002389714	4.94E-10
cg19656070	TAX1BP3(+18)	17	3571978	Promoter	0.01069978	0.001727363	5.85E-10
cg07283595	DHX33(+400)	17	5372003	Promoter	0.023494356	0.003797213	6.12E-10
cg25757017	OLR1(+18303)	12	10306462	Promoter, Enhancer	0.021080747	0.00341288	6.54E-10
cg02286335	ZWINT(-11)	10	58121068	Promoter	0.0149277	0.002433753	8.59E-10
cg15248577	,SFMBT2(+3015)	10	7450456	Promoter	0.013766093	0.002252311	9.84E-10
cg14078059	GNS(-21459)	12	65174660	Enhancer	0.016388831	0.0026961	1.21E-09
cg04875007	RPS2(-21)	16	2014871	Promoter	0.012867742	0.002125921	1.42E-09
cg18764107	LBR(+169)	1	225615669	Promoter	0.012219992	0.002018668	1.42E-09
cg04759220	JMY(+660)	5	78532560	-	0.014868421	0.002458298	1.46E-09
cg08522087	ANKH(0)	5	14871910	-	0.007733637	0.00128734	1.88E-09
cg13579901	DCAF6(-280)	1	167905492	Promoter	0.022337576	0.003720867	1.93E-09

Supplemental table. Continued.

CpG	Nearest gene (+/- base pairs)	Chr	Base pairs	Location	Beta	Standard error	P-value
cg02750935	PRELID2(-59)	5	145214981	Promoter, Enhancer	0.007716834	0.001285997	1.97E-09
cg05234169	CCDC59(-78)	12	82752252	Promoter	0.016282794	0.002714563	1.99E-09
cg05884522	NOL7(-44)	6	13615538	Promoter	0.01307324	0.00218602	2.23E-09
cg06091566	SAMD11(-523)	1	860621	-	0.026435441	0.004423976	2.29E-09
cg24079591	MARK3(-213)	14	103851511	Promoter	0.015991172	0.002680227	2.43E-09
cg11220663	ADD2(+7)	2	70994863	-	0.022700901	0.003806692	2.47E-09
cg12043722	CORO2B(-38066)	15	68870836	-	0.021346312	0.003589317	2.73E-09
cg07262328	CD44(+269)	11	35160709	Promoter	0.013942252	0.00235062	3.01E-09
cg16163324	RAD54L(-458)	1	46712932	Promoter	0.030437342	0.005137256	3.13E-09
cg23222745	-	6	27791912	-	0.016727733	0.002826838	3.27E-09
cg03243965	HSH2D(+9703)	19	16254516	Promoter	0.009874424	0.001670143	3.37E-09
cg06506598	ZFYVE16(+80028)	5	79783889	Promoter	0.024948733	0.004222809	3.46E-09
cg16370446	FAM200B(-43)	4	15683284	Promoter	0.010397718	0.001763573	3.73E-09
cg02547025	LBH(-97)	2	30454275	Promoter	0.008294564	0.001407597	3.80E-09
cg25335190	-	6	27791899	-	0.01905164	0.003241695	4.18E-09
cg22270384	ASNA1(-41)	19	12848240	Promoter	0.02699067	0.004627302	5.45E-09
cg21028463	MFSD11(+124)	17	74733682	Promoter	0.011167499	0.001916108	5.60E-09
cg15698851	TMEM66(+281)	8	29940391	Promoter	0.014332978	0.002463582	5.96E-09
cg15965583	ENSA(+172)	1	150601949	Promoter	0.025400177	0.004366307	5.98E-09
cg22100652	TRIM27(+57387)	6	28834404	Promoter	-0.026777751	0.004614584	6.52E-09
cg08655589	SLC6A6(+46)	3	14444175	Promoter	0.014234875	0.002454671	6.67E-09
cg17834180	IKZF5(+94)	10	124768240	Promoter	0.015030674	0.002593684	6.83E-09
cg10101470	LRRC8D(-870)	1	90286633	Promoter	0.00739195	0.001276564	7.02E-09
cg21597684	HECW2(-42)	2	197457400	-	0.015548919	0.002685279	7.02E-09
cg19273746	B3GAT2(+700)	6	71666063	Promoter	0.023624873	0.004104604	8.63E-09
cg22870667	-	8	521182	Enhancer	-0.027723903	0.004826716	9.26E-09
cg20718350	ASCL1(+819)	12	103352294	-	0.009430784	0.001644405	9.75E-09
cg18289490	SPATA2(+33)	20	48532070	Promoter	0.013736303	0.002396973	1.00E-08
cg20462855	HEY2(-370)	6	126070385	-	0.0072723	0.001269581	1.02E-08
cg20297566	TBC1D23(+267)	3	99979976	Promoter	0.031187463	0.005450513	1.05E-08
cg08709073	RGS7BP(-243)	5	63802184	-	0.018571888	0.003250119	1.10E-08
cg19080138	HERPUD2(+14)	7	35734733	Promoter	0.013942223	0.002443189	1.15E-08
cg26580332	-	8	143919495	-	0.018298842	0.003209963	1.19E-08
cg03713379	SLC12A2(+117789)	5	127537295	-	0.016365348	0.002875992	1.27E-08

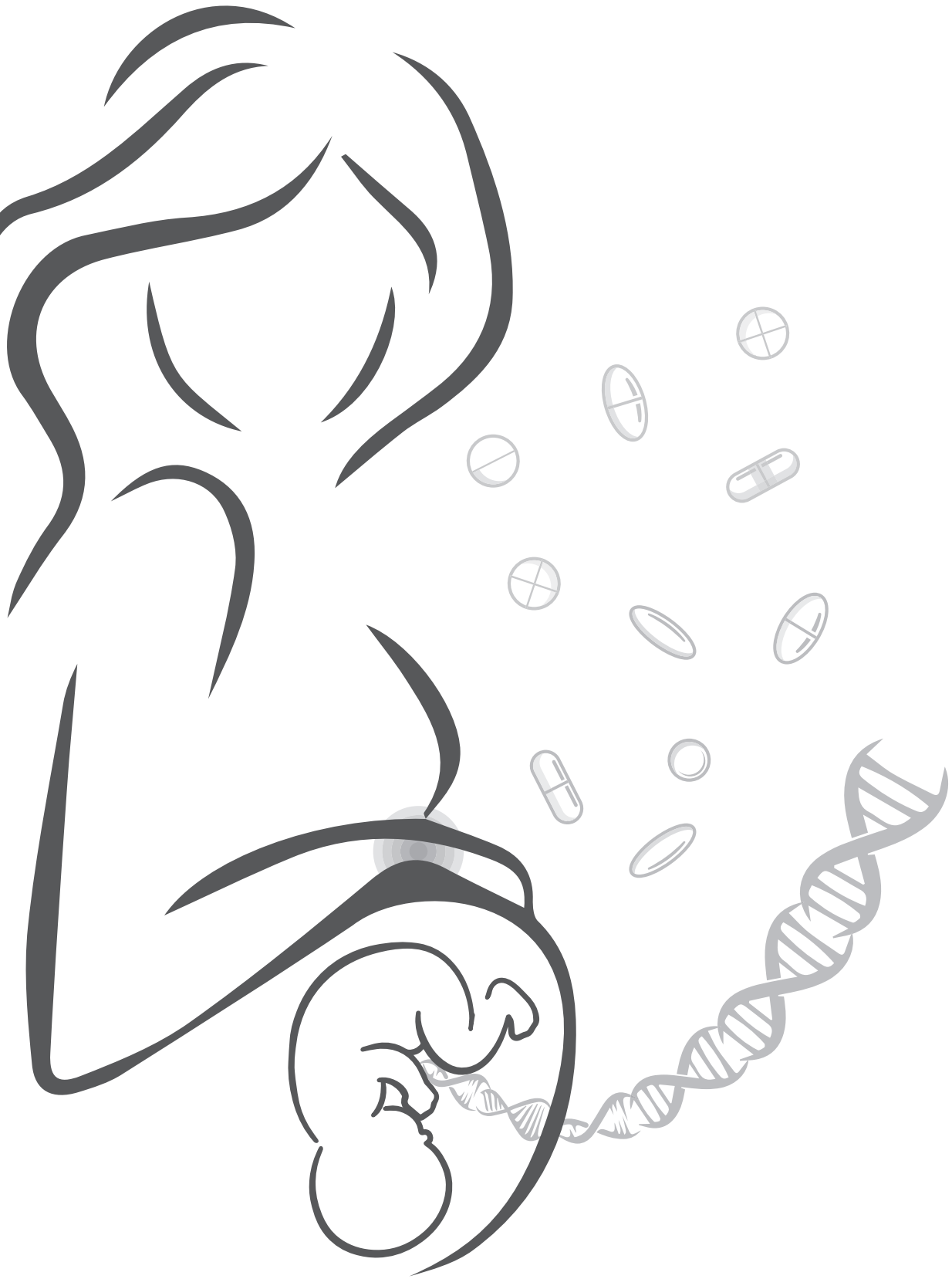
Supplemental table. Continued.

CpG	Nearest gene (+/- base pairs)	Chr	Base pairs	Location	Beta	Standard error	P-value
cg18120578	E2F7(-24)	12	77459407	Promoter	0.014961422	0.002635655	1.37E-08
cg02994863	PGM1(-29613)	1	64059297	Promoter	0.013618631	0.002400823	1.41E-08
cg15408407	RPS15(+100)	19	1438438	Promoter	0.006751614	0.001191922	1.47E-08
cg21243939	SAMD4A(-1168)	14	55033137	-	0.015308476	0.002707084	1.56E-08
cg04041942	INTS9(-158)	8	28747613	Promoter	0.01474484	0.002608622	1.58E-08
cg12360123	-	10	79984532	Enhancer	-0.043318426	0.007667128	1.61E-08
cg14229247	ANP32B(-373)	9	100745139	Promoter	0.005090587	0.000901141	1.61E-08
cg05944369	HMGB2(-673)	4	174255616	Promoter	0.017054023	0.003024432	1.71E-08
cg04335562	ZNF77(-56)	19	2945000	Promoter	0.023036961	0.004086777	1.73E-08
cg15012981	MAD2L2(+10644)	1	11741009	Promoter	0.005040813	0.000896171	1.86E-08
cg14225021	ITGB6(-69870)	2	161126482	Promoter, Enhancer	0.011800981	0.002102176	1.98E-08
cg09572053	C6orf1(-143622)	6	34360501	Promoter	0.006279856	0.001121122	2.13E-08
cg26646903	TBCCD1(-92)	3	186285208	Promoter	0.011590163	0.002084438	2.69E-08
cg13841783	-	6	32293117	-	-0.030474363	0.00548179	2.71E-08
cg11706469	FGF8(+420)	10	103535362	-	0.027519708	0.004959609	2.88E-08
cg13425677	MAP3K1(+1215)	5	56112090	-	0.014548024	0.0026336	3.31E-08
cg15642758	HDGFRP3(-268)	15	83876612	Promoter	0.013003375	0.002354288	3.33E-08
cg06812693	RBPJ(+794)	4	26323246	-	0.020035197	0.003629075	3.38E-08
cg11336323	-	19	41946040	Promoter	-0.03675832	0.00666397	3.47E-08
cg18913103	FBXW7(-1391)	4	153457599	Promoter	0.016671965	0.003022876	3.48E-08
cg10393416	PLK4(+86)	4	128802077	Promoter	0.011204472	0.002035668	3.71E-08
cg20692684	EPC1(-99070)	10	32735158	Promoter	0.013607155	0.002480731	4.13E-08
cg04551440	KATNAL1(-8)	13	30881194	Promoter	0.017295098	0.003153543	4.15E-08
cg04738301	NTN4(-21)	12	96184580	-	0.006624531	0.001210765	4.47E-08
cg01374398	SCAP(+493)	3	47516975	Promoter	0.02248462	0.004109964	4.48E-08
cg05740879	CDKN1C(+185152)	11	2721866	-	-0.027389961	0.005036612	5.38E-08
cg09636406	RECQL5(+110)	17	73663134	Promoter	0.013516309	0.002488052	5.56E-08
cg10391895	ARL2(+112)	11	64781672	Promoter	0.016185438	0.002980622	5.63E-08
cg07361491	GATA6(+96)	18	19749535	-	0.011950742	0.002201116	5.65E-08
cg23055081	TRIM26(-21)	6	30181315	Promoter	0.019228906	0.003543944	5.77E-08
cg15040188	-	19	621137	-	-0.025437993	0.004696691	6.09E-08
cg07226281	MAPK8IP3(+378)	16	1756622	Promoter	0.009645234	0.001783131	6.33E-08
cg17364044	PELI1(+378)	2	64371202	Promoter	0.012546186	0.00232473	6.78E-08
cg12260146	PDCD6IP(+196)	3	33840237	Promoter	0.030045517	0.005570297	6.90E-08

Supplemental table. Continued.

CpG	Nearest gene (+/- base pairs)	Chr	Base pairs	Location	Beta	Standard error	P-value
cg24906202	NEO1(+412)	15	73345212	-	0.013867409	0.002573678	7.12E-08
cg06803925	CENPL(+167)	1	173793126	Promoter	0.009030316	0.001677537	7.32E-08
cg16485682	GATA6(+101)	18	19749540	-	0.006185935	0.001149349	7.36E-08
cg23681311	MAPK1(+67)	22	22221878	Promoter	0.008424076	0.001567243	7.65E-08
cg23243902	SIDT2(-113)	11	117049849	-	0.022369089	0.004171023	8.19E-08
cg19906131	PANX1(+447)	11	93862516	-	0.011899087	0.002224471	8.84E-08
cg25668236	ST8SIA4(+2169)	5	100236841	-	0.010861389	0.002033066	9.17E-08
cg21475747	HNRNPAB(-58)	5	177631425	Promoter	0.012719026	0.00238298	9.43E-08
cg26021273	MNX1(-10742)	7	156814112	-	0.020861613	0.003918929	1.02E-07
cg05300158	SETD7(-127)	4	140477727	-	0.008149892	0.00153159	1.03E-07
cg02613964	-	3	44690321	Promoter	-0.019188607	0.003608882	1.05E-07
cg04678743	COPG2(+106)	7	130353515	Promoter	0.011250205	0.00211627	1.06E-07

bp=base pair; Chr= chromosome; SE= standard error
[$] Beta represents the difference in DNA methylation at the given CpG site in children born to mothers with RA(FEPRA-study) as compared with children born to mothers from the general population(Generation R Study)
[#] Location in promoter, enhancer or unknown(-)



Associations between antenatal prednisone exposure and long-term cortisol and cortisone concentrations in children born to women with rheumatoid arthritis; results from a nationwide prospective cohort study

Hilal Ince-Aşkan
Erica L.T. van den Akker
Yolanda B. de Rijke
Elisabeth F.C. van Rossum
Johanna M.W. Hazes
Radboud J.E.M. Dolhain

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ABSTRACT

Objectives. To identify whether children with antenatal prednisone exposure have chronically elevated cortisol and cortisone concentrations, an altered body composition, or higher blood pressure. In addition, to identify whether maternal rheumatoid arthritis disease(RA) activity is associated with these alterations.

Methods. In this prospective study, 56 children(mean age=10.0 years) with, and 61 children(mean age=9.6 years) without antenatal prednisone exposure, born to women with RA, were included. Hair cortisol and cortisone were analyzed using liquid chromatography–tandem mass spectrometry. Linear regression models were built to analyze differences between the two groups, corrected for relevant covariates. Hair cortisol concentrations were also compared between the study population and an age-matched healthy reference group(N=150 children, mean age=9.8 years).

Results. Hair cortisol and cortisone concentrations were similar in children with and without antenatal prednisone exposure(median cortisol 1.14pg/mg[IQR 0.67 -1.75] and 1.15pg/mg[IQR 0.65 – 2.21], and median cortisone 6.76pg/mg[IQR 5.42 – 8.86] and 7.40pg/mg[IQR 5.39 – 10.73], respectively). Antenatal prednisone exposure and maternal RA disease activity were also not associated with body composition or blood pressure. Hair cortisol concentrations were not different in children born to mothers with RA compared with children from the reference group.

Conclusion. This, in its kind, large and unique long-term prospective study demonstrates that low-dose antenatal prednisone exposure and maternal RA disease activity are not associated with negative consequences in prepubertal childhood. The findings of this study are reassuring and support the assumption that low-dose maternal prednisone use during pregnancy is safe for the offspring, at least until the age of approximately 10 years.

Ideally during pregnancy women would not use any medication, however sometimes it's inevitable, like the use of corticosteroids in autoimmune disorders. In animal studies it has been shown that an excess of maternal glucocorticoids during pregnancy, for example administration of synthetic glucocorticoids, is associated with altered hypothalamic-pituitary-adrenal (HPA) axis activity with higher plasma cortisol levels of the offspring, higher blood pressure,hyperglycemia, and visceral obesity in later life[1-7].

There are also several human studies demonstrating that adverse circumstances during pregnancy, that are related with cortisol concentrations in the mother (e.g. maternal stress[8], maternal depression[9-11], and maternal anxiety during pregnancy[12-14]), result in elevated cortisol concentrations and an altered cortisol response in the offspring. Chronically elevated cortisol levels are associated with an increased risk of abdominal obesity, hypertension, non-insulin dependent diabetes mellitus and cardiovascular disease[15-20].

Maternal cortisol rises throughout pregnancy, but normally only 10-20% reaches the fetus due to the oxidation of cortisol to cortisone by 11beta-hydroxysteriod dehydrogenase type II (11βHSD2) enzyme in the placenta[21-23]. Cortisone can be reverse metabolized into cortisol[24]. The 11βHSD2enzyme also converts the active prednisolone into its inactive metabolite prednisone[25]. Despite the presence of this enzyme, children born to mothers who use prednisone during pregnancy could be exposed to higher concentrations of corticosteroids in utero in at least three different ways.

First, since the placenta is not fully developed before 12 weeks of gestation, in that period passive diffusion of prednisolone to the fetus takes place[26]. Second, the capacity of the 11βHSD2 enzyme may be exceeded by the external administration of corticosteroids[26, 27], resulting in increased placental availability of prednisolone. Finally, the activity of 11βHSD2 is down-regulated by the pro-inflammatory cytokines Tumor Necrosis Factor (TNF), Interleukin (IL)-1 and IL-6, which are elevated in autoimmune disorders like Rheumatoid Arthritis (RA)[26, 28], resulting in decreased inactivation and increased availability of prednisolone.

Exposure to higher corticosteroid concentrations in utero may influence the development of the fetal HPA axis, with reduced negative feedback due to reduction in hippocampal glucocorticoid receptor expression[2]. As a result, this may lead to an overactive HPA axis, and elevated circulating glucocorticoids in the offspring[2].

De Steenwinkel et al.[26] reported that children born to mothers with RA who used prednisone during pregnancy had higher salivary cortisol levels at the age of approximately 7 years, independent of RA disease activity of the mother during pregnancy[26]. However, there was no association with physical signs of higher cortisol levels. The main limitation of that study is that the analysis of cortisol was from a single day saliva sample collection, which is a useful method to study acute HPA axis reactivity, but is not a good reflection of long-term cortisol levels[29, 30].

Since then, a new method has become available to measure both long-term cortisol and cortisone concentrations in scalp hair[31]. Several studies have shown that this is a reliable, non-invasive method to measure endogenous cortisol and cortisone, in adults[32-35], and in children[30, 36, 37]. Hair cortisol is a reflects long-term cortisol exposure[30]. It has been shown that obese children have higher hair cortisol concentrations compared with non-obese children[24, 30]. Furthermore, in a large population based cohort, hair cortisol concentrations were already associated with BMI and adverse body fat distribution in children at young age (mean age of 6 years)[38]. Also, children's hair cortisol levels have been shown to be associated with stress at school entry[37].

Hair cortisol and cortisone analysis could provide more information about the long-term consequences of antenatal prednisone exposure.

In the current study, we investigated whether children with antenatal prednisone exposure have signs of altered HPA axis activity, reflected by chronically elevated hair cortisol and cortisone concentrations, an altered body composition (risk factors for future cardiovascular and metabolic disease), or higher blood pressure compared with children without antenatal prednisone exposure. In addition, since pro-inflammatory cytokines may cause downregulation of 11bHSD followed by increased transfer of prednisolone to the fetus, we analysed the associations between maternal RA disease activity during pregnancy and the earlier mentioned outcome measures in childhood.

METHODS

Study Population

In a previous study[26] that showed that antenatal prednisone exposure is associated with elevated cortisol concentrations in saliva at the age of approximately 7 years, 42 children with and 63 children without prednisone exposure were included. Hair cortisol concentrations correlate with long-term salivary cortisol production measured over a one-month period[32]. Therefore, in order to acquire enough power in this prospective cohort study, we aimed to include comparable numbers of participants. In this study, a total of 56 children with (prednisone dose ≥5mg/day, during at least 2 trimesters of pregnancy) and 61 children without antenatal prednisone exposure born to women with RA , aged 5 to 15 years, were included between 2014 and 2018.

First, children born to women who participated in the Pregnancy induced Amelioration of Rheumatoid Arthritis (PARA) study were included, a nationwide prospective cohort study on RA and pregnancy and onwards from the Netherlands[39]. The PARA-study has been described in detail elsewhere[39]. In the PARA-study, RA patients who met the 1987 revised American College of Rheumatology criteria[40] with a wish to conceive or already pregnant,

were enrolled between 2002 and 2008 (last visit in 2010).

Second, siblings of the children born to PARA-participants were included (if their mother was already diagnosed with RA before pregnancy). Finally, after the inclusion was not sufficient, children born to women with RA who visited the outpatient clinic of the department of Rheumatology in the Erasmus MC Rotterdam, the Netherlands, were included to accomplish the intended number of inclusions. At this outpatient clinic, RA disease activity and medication use during pregnancy are properly recorded.

In total, 97 children born the PARA-study participants, 14 siblings and 6 children born to women who visited the outpatient clinic were included. Exclusion criteria were: congenital abnormalities, chronic illness of the child, non-Caucasian ethnicity, scalp hair shorter than 2 cm.

Reference population

As a reference population, 252 healthy children born to women from the general population were used. These children had been recruited, as described in more detail elsewhere[41]. Briefly, children were eligible for inclusion if they were between the age of 4 and 18 years, did not use glucocorticoids 3 months prior to or during the study, and did not suffer a chronic disease. Healthy controls were recruited from primary and secondary schools in the Netherlands and among siblings of children attending the pediatric outpatient clinic[41]. For comparison with the current study, only data on Caucasian children between the age of 5 and 15 years was used, resulting in 150 participants in the reference group.

Data collection

Data collection during pregnancy

In the PARA-study participants were visited at their home address before pregnancy (if possible), three times during pregnancy and three times after the birth of the child (at 6, 12 and 26 weeks). At all time-points data on mother and child was collected. RA disease activity was measured using the Disease Activity Score based upon 3 variables; swelling and tenderness by palpation in 28 joints and a C-Reactive Protein (DAS28-CRP(3)). For participants whose mother did not participate in the PARA-study, information on their disease activity and medication use during pregnancy, pregnancy course, and pregnancy outcome (from 1 year before delivery until 1 year after delivery) was obtained from their rheumatologist and gynecologist.

Hair collection

Participants were visited at their home address once during the study period. During this visit, a lock of 3 cm with approximately 100 hairs was cut from the posterior vertex of the child's scalp, as close to the scalp as possible. There is a wide consensus that proximal parts of the hair reliably reflect chronic cortisol concentrations[42]. Exogenous environmental factors, such as frequent hair washing and cosmetic treatments could decrease cortisol levels in the more distal (older) segments of the hair[31, 42-44]. Additionally, sampling of hair that is taken from the posterior vertex is the most optimal, because the intra-individual variation is smallest at that site[31, 34]. Hair growth patterns vary across different regions of the scalp and the posterior vertex region shows the most uniform growth rates[45]. Because hair grows approximately 1 cm per month, a hair sample of 1 cm is thought to reflect the mean exposure of cortisol in 1 month[30, 46].

The hair samples were taped to a piece of paper, and the proximal side was marked. The hair samples were stored at room temperature in an envelope until analysis.

Parents were also asked to fill out a questionnaire on e.g. frequency of washing the hair, hair color, cosmetic hair treatments and medication/illness of their children.

Anthropometry

During the visit, blood pressure, growth and body composition were also measured (height, weight, hip and waist circumference, and skin folds using Holtain caliper). All measurements, except for weight, were performed 3 times. Blood pressure was measured 3 times in rest with an automatic device (Omron M6 AC; Omron Healthcare, Hoofddorp, the Netherlands), using a cuff appropriate to the size of the child's upper arm. The mean of the 3 measurements were used in the analysis. Fat percentage was calculated using the skin folds (biceps, triceps, subscapular, and suprailiacal) with the Durnin and Womersley formula[47].

Body mass index (BMI) was calculated using the formula weight (kg)/height^2(m^2). Systolic and diastolic blood pressure/height ratios were calculated, since those ratios have been shown to correlate with the corresponding blood pressure percentiles in both male and female children[48]. All other child-related values were transformed into standard deviation scores (SDS) for age and gender according to the 2010 Dutch reference values[49], using the Growth Analyser(version 4.0; Growth analyser BV, Rotterdam, the Netherlands, www.growthanalyser.org). Birth weight was expressed as birth weight SDS, corrected for gestational age and gender[50].

Data collection reference population

Hair samples and data on anthropometrics were collected during school visitation. Demographic information, data on general health, the use of medication, and hair care were collected through questionnaires[41]. Hair collection was performed with a similar method as in the current study.

Cortisol and cortisone analysis in hair

The hair samples were processed and analysed, by liquid chromatography–tandem mass spectrometry (LC-MS/MS) as described previously[44], at the department of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, The Netherlands.

Statistical Analysis

For all subjects descriptive statistics were calculated as numbers, percentages, means, medians, standard deviation scores (SDSs) and interquartile ranges (IQRs). For comparing baseline characteristics, Student t tests were used for normally distributed continuous variables, Wilcoxon rank-sum tests for not normally distributed continuous variables, and Chi squared and Fisher's exact tests for categorical variables. Cortisol and cortisone were logarithmically (log10) transformed to achieve a normal distribution. Linear regression models were built to analyse associations between cortisol and cortisone, and antenatal prednisone exposure. Factors associated in other studies[24, 51] with hair cortisol and cortisone concentrations (age, sex, socioeconomic status[SES] based on the educational level of the mother, season of hair collection, hair color, washing frequency), were used as independent covariables. Similarly, a regression model was built to analyse the association between maternal DAS28-CRP(3) during pregnancy and cortisol and cortisone concentrations. Furthermore, associations between cortisol, cortisone, DAS28-CRP(3) and the anthropometric measurements (BMI SDS, fat percentage SDS based on skinfolds, waist and hip circumference SDS, systolic and diastolic bloodpressure SDS, ratio waist-hip circumference, and ratio skinfolds trunk to skinfolds peripheral) were analysed using linear regression.

In addition, a linear regression analysis was performed to compare the cortisol concentrations children born to mothers with RA, with the reference population using stepwise backwards selection of relevant covariates (age, sex, weight SDS, height SDS, BMI SDS, waist circumference SDS, height circumference SDS, SES, washing frequency, and hair color).

P-values ≤0.05 were considered statistically significant. All statistical analysis were performed using STATA software version 15.1 for Windows.

Ethics

This study is in compliance with the Helsinki Declaration. The Medical Ethics Committee at the Erasmus MC University Medical Center Rotterdam, the Netherlands, approved the current study (MEC-2014-395) and the PARA-study (MEC-214.320/2002/117). Parents(and children) received oral and written information about the study. Informed consent was signed by both parents, and their children if they were aged ≥12 years.

RESULTS

Participants

In total, 252 children born to mothers with RA and both of their parents were invited to participate in this study (figure 1). From these, 58 (23.0%) did not respond to the invitation and could not be reached by mail or phone. From the 72 invited subjects who refused participation, 35 (48.6%) did not provide a reason, 16 (22.2%) explained that they had participated in too many studies, and 12 (16.7%) were not interested in participation due to the fact that this study had to be performed in their children. After exclusion of 5 children with antenatal prednisone exposure <5mg/day and/or less than during 2 trimesters, in total 117 children were included in this study. From these, we included 56 children who had antenatally been exposed to prednisone (prednisone dose ≥5mg/day, during at least 2 trimesters of pregnancy) and 61 children who had not been exposed.

Participants and non-participants were compared. There were no statistically significant differences in maternal DAS28-CRP(3) in 3rd trimester, maternal age at delivery, presence of autoantibodies in mother, SES based on educational level of the mother, sex of the offspring, and birth weight. Gestational age was borderline significant (p=0.05), with a median gestational age of 277.9 days (IQR 270.9 – 286.0) in participants and 275.0 days (IQR 268.0 – 282.5) in non-participants.

Descriptive statistics of the study population are shown in table 1. Overall, the baseline characteristics were comparable in the prednisone and the non-prednisone group. Birth weight and gestational age were both significantly lower in the group with prednisone exposure compared with children without prednisone exposure. Mean birth weight was 3167.5g (sd=691.9) in the prednisone group vs 3435.4g (sd=584.2) in the non-prednisone group, p-value 0.01. The median gestational age was 273.0 days (IQR 258.0 – 281) in the prednisone vs 281.0 days (IQR 274.0 – 287.0) in the non-prednisone group, p-value<0.001. Birth weight corrected for gestational age and sex was not different between the two groups. Maternal characteristics were also comparable in both groups, except for the DAS28-CRP(3), which was higher in the prednisone group, 3.7 (sd=1.1) vs 2.8 (sd=0.9) in the non-prednisone group, p-value <0.001. Detailed information on descriptive statistics of the children are provided in supplemental table 1.

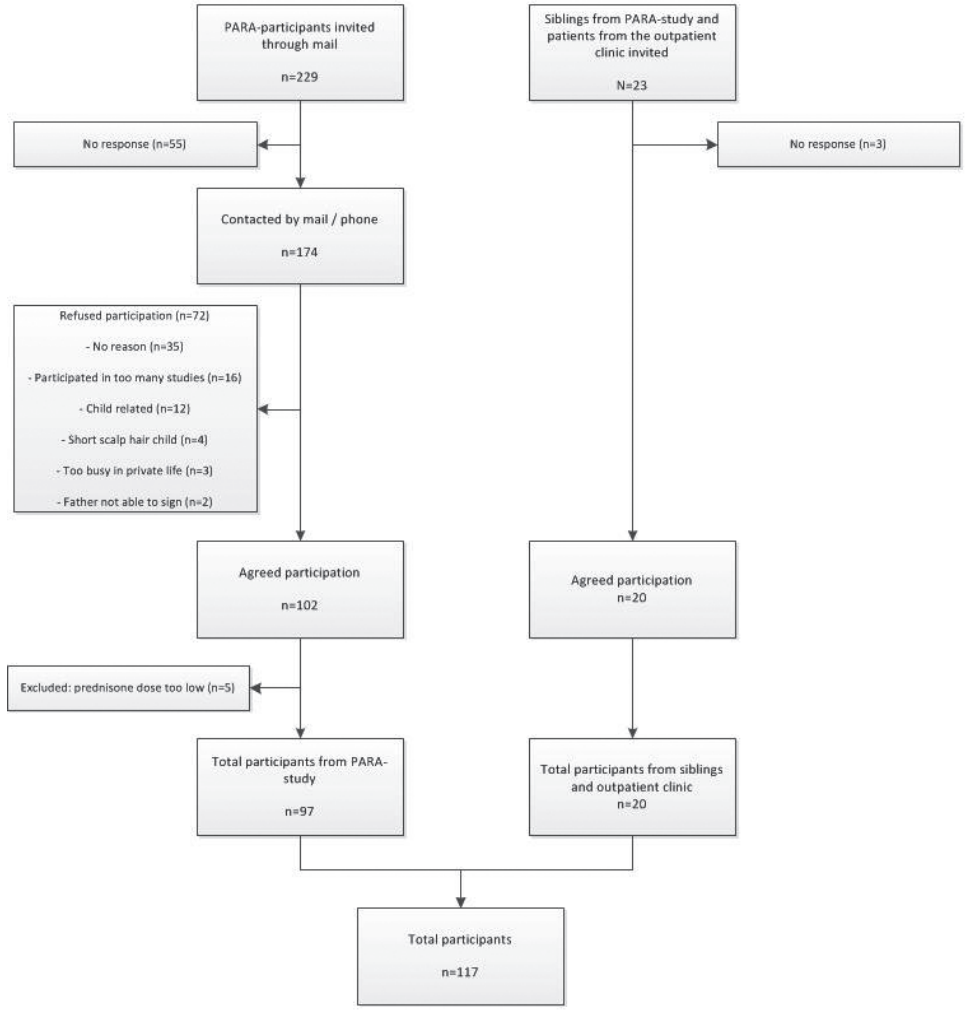


Figure 1. Flow-chart of the study population and inclusion of study participants.

Table 1. Descriptive statistics of study population

	Prednisone (N=56)	Non-prednisone (N=61)	Reference population (n=150)
Age child, yrs, mean (sd)	10.0 (2.5)	9.6 (2.2)	9.8 (3.1)
Male	36 (64.3)	34 (55.7)	68 (45.3)*
Birth weight g, mean (sd)	3167.5 (691.9)*	3435.4 (584.2)*	-
Birth weigth sds, mean (sd)	-0.1 (1.0)	-0.2 (1.2)	-
Gestational age days, median(IQR)	273.0 (258.0 – 281)**	281.0 (274.0 – 287.0)**	-
SES based on educational level of the mother			
middle	15 (26.8)	23 (38.3)	21 (14.0)
high	35 (62.5)	33 (55.0)	99 (66.0)
Black/brown hair color	16 (28.6)	15 (24.6)	47 (31.3)**
Washing frequency (per week) ≥3	25 (44.6)	25 (41.0)	52 (34.7)**
Season hair collection winter & fall	39 (69.6)	37 (60.7)	
Maternal DAS28-CRP(3) in 3rd trim, mean (sd)	3.7 (1.1)**	2.8 (0.9)**	
Maternal age at delivery, yrs, mean (sd)	32.9 (3.8)	32.9 (3.9)	
Maternal autoantibodies			
Present	33 (58.9)	35 (57.4)	
Absent	8 (14.3)	17 (27.9)	
Unknown	14 (25.0)	9 (14.8)	

* p-values <0.05 **p-values <0.001, statistics were performed comparing the prednisone and non-prednisone group, and the prednisone and non-prednisone group together versus the reference population. sd=standard deviation; sds=standard deviation scores; IQR=interquartile range; SES=socioeconomic status; BP=blood pressure; DAS28-CRP(3)=disease activity score in 28 joints using C-reactive protein levels

Associations between antenatal prednisone exposure and cortisol and cortisone concentrations in scalp hair

The median hair cortisol concentration was 1.14 pg/mg (IQR 0.67 -1.75) in the children with antenatal prednisone exposure, and 1.15 pg/mg (IQR 0.65 – 2.21) in the children without antenatal prednisone exposure. The median hair cortisone concentration was 6.76 pg/mg (IQR 5.42 – 8.86) in the prednisone group, and 7.40 pg/mg (IQR 5.39 – 10.73) in the non-prednisone group. After log-transformation, cortisol and cortisone were normally distributed. Hair cortisol and cortisone concentrations were not significantly different between the two groups.

In our study population 17 children were exposed to prednisone with a mean dose per day ≥10.0 mg. Repeating the analyses in these children compared with the children without antenatal prednisone exposure did not result in significant associations between prednisone exposure and hair cortisol and cortisone concentrations (p-value>0.30). The median cortisol and cortisone concentrations were 0.97 pg/mg (IQR 0.68 – 1.57) and 6.26

(IQR 4.80-8.49) in the prednisone group (≥10.0mg daily), and 1.15 (IQR 0.65 – 2.21) and 7.40 (IQR 5.39 – 10.73) in the non-prednisone group, respectively.

In univariable analysis, a higher SES (based on the educational level of the mother) was associated with higher hair cortisol and cortisone concentrations, and a washing frequency ≥3 times with lower cortisol concentrations (Table 3). Children born to mothers with a higher SES had a lower washing frequency of their hair. The association between SES and levels of cortisol and cortisone was not present in the multivariable model.

In the multivariable linear regression models, prednisone exposure was not associated with hair cortisol and cortisone concentrations. The median of the mean prednisone dose per day during pregnancy was 7.5mg/day (IQR 5.0 -10.0). The mean prednisone dose and prednisone exposure in the first trimester were also not associated with these concentrations. In the multivariable models, only washing frequency was significantly associated with the levels of cortisone in hair. A washing frequency ≥3 times per week was associated with lower cortisone concentrations (p-values<0.05). Age, sex, season of hair collection, and hair color were not associated with hair cortisol and cortisone in the uni- and multivariable models.

Associations between antenatal prednisone exposure and anthropometric values

Antenatal prednisone exposure was not associated with the anthropometric measurements (BMI SDS, fat percentage SDS, waist circumference SDS, hip circumference SDS, ratio waist-hip, ratio systolic blood pressure/height, ratio diastolic blood pressure/height, and the ratio skinfolds trunk/peripheral), corrected for age, sex, and SES (based on the educational level of the mother). The anthropometric values were also not associated with the hair cortisol and cortisone concentrations.

Associations with maternal RA disease activity

Maternal RA disease activity in the third trimester was not associated with hair cortisol and cortisone concentrations in the children. Maternal DAS28-CRP(3) in the third trimester was also not associated with the anthropometric measurements.

Comparison with reference population

The median hair cortisol concentration in the reference population was 1.93 pg/mg (IQR 1.18 -3.11). After log-transformation, cortisol was normally distributed.

Hair cortisol concentrations were not different between children born to mothers with RA and the reference population, corrected for the relevant variables mentioned previously, in a multivariate linear regression model. In this model, an older age of the child and higher weight SDS were significantly associated with higher cortisol levels (p-values <0.05).

DISCUSSION

Prednisone is an anti-inflammatory drug that is prescribed to patients with an autoimmune disease, e.g. RA, also during pregnancy. Research into the long-term side effects of prednisone use during pregnancy on the offspring has been limited. In this relatively large and unique nationwide study, we followed the majority of the mothers and their children prospectively during and after pregnancy. The results of our study show that children with low-dose antenatal prednisone exposure do not have chronically elevated cortisol and cortisone concentrations in hair, compared with children without antenatal prednisone exposure. This is the first study to show these results prospectively from the antenatal period onwards in a (for this design) large cohort using scalp hair, reflecting chronic cortisol and cortisone concentrations. Adjustment for confounders allowed an independent analysis of the effects of antenatal prednisone exposure in childhood.

Prednisone exposure was also not associated with anthropometric measurements like BMI SDS, fat percentage SDS, waist circumference SDS, hip circumference SDS, ratio waist-hip, and the ratio skinfolds trunk/peripheral, or with ratio systolic blood pressure/height and ratio diastolic blood pressure/height. Furthermore, maternal RA disease activity during pregnancy was not associated with cortisol and cortisone concentrations, and anthropometric measurements. Children born to mothers with RA had a similar cortisol concentration compared with the reference population.

In general, antenatal exposure to glucocorticoids might result in chronically elevated cortisol and cortisone concentrations in the offspring[1-4, 46]. Previous studies, mostly animal but also in humans, demonstrated long-term effects of maternal stress[8], maternal depression[9, 10], and maternal anxiety during pregnancy[12-14] on cortisol concentrations or cortisol responses in offsprings. As mentioned before, adverse events during pregnancy have been shown to influence the development of the fetal HPA axis. The systemic inflammation in RA patients, and medication use during pregnancy are also considered as an adverse event during pregnancy. This is one of the few prospective studies in humans studying offspring antenatally exposed to synthetic glucocorticoids with the most reliable method to measure chronic cortisol and cortisone concentrations (in scalp hair)[33]. In our current study we did not find any association between antenatal exposure to low-dose prednisone, maternal RA disease activity during pregnancy and long-term cortisol and cortisone concentrations or with an altered body composition or higher blood pressure in childhood.

In a previous study by de Steenwinkel et al.[26], children born to mothers with RA with antenatal prednisone exposure had higher daytime cortisol levels (measured in saliva) compared with children without prednisone exposure. However, measuring cortisol in saliva is not a reliable method for long-term concentrations due to the circadian rhythm, pulsatile secretion, daily variation and reactivity to acute (transient) stress[21, 29, 30]. Measuring cortisol in hair reliably reflects chronic concentrations. The elevated salivary cortisol levels in children with antenatal prednisone exposure born to mothers with RA from the study by de Steenwinkel et al.[26] could be a result of increased HPA axis reactivity. However, for studying HPA axis reactivity, cortisol concentrations should preferably be measured before and after administrating a stressor. In another study by de Steenwinkel et al.[52], maternal RA disease activity during pregnancy and antenatal prednisone exposure had no influence on the body composition or on the risk for the metabolic syndrome of approximately 7-years-old offspring[52]. In concordance with this study, we did not find either any associations between maternal RA disease activity during pregnancy, antenatal prednisone exposure, and body composition in offspring aged approximately 10 years, which is reassuring. It is still possible that the consequences for these offsprings will emerge later in their life, therefore following these children until post-pubertal age is interesting for future research. The chronic use of prednisone and high RA disease activity are associated with reduced bone mineral density (BMD)[53, 54]. In our study, we did not explore the effect of antenatal prednisone exposure on the BMD of the offspring. However, in a previous study by de Steenwinkel et al.[55], they investigated the influence of medication use and disease activity of the mother during pregnancy on the offspring at the age of approximately 7 years. In that study, a dual x-ray absorptiometry scan was performed in 108 children born to women with RA. They did not find any association between BMD and prednisone use or RA disease activity during pregnancy, even after correcting for all known associated variables, which is reassuring.

Currently, the treatment options for RA during pregnancy are prednisone, sulfasalazine, hydroxychloroquine and TNF inhibitors[56]. Most patients start with sulfasalazine or hydroxychloroquine, and when they do not respond to this therapy, prednisone or TNF-inhibitors are added. Compared with prednisone, TNF inhibitors are expensive and worldwide not easily available for all patients with RA. Therefore, it is important that this study showed the long-term safety of maternal prednisone use during pregnancy.

Certain limitations should be taken into account when interpreting the results of this study. First, from the 252 invited children (and their parents), 122 (48.4%) were willing to participate in this study. In total, 72 (28.6%) refused participation. Baseline characteristics were comparable between participants and non-participants. These findings decrease the likelihood that selection bias influenced the results of this study.

Second, in this study, the median of the mean prednisone dose per day during pregnancy was 7.5 mg (IQR 5.0 -10.0). The use of prednisone concentrations above 7.5 mg/day or even 10 mg/day might be more likely to influence the long-term cortisol and cortisone concentrations in the offspring. However, repeating the analyses in the children who were antenatally exposed to higher prednisone dosages (mean dose per day ≥10.0 mg), did not lead to different results. In addition, lower concentrations of prednisone are often sufficient for the treatment of RA during pregnancy. Unfortunately, we did not have information on

maternal weight during pregnancy for the majority of the participants. Maternal weight during the visit when the children's hair was collected (child age approximately 10 years) was measured. However, since that would not be completely representative of the weight during pregnancy, prednisone dosages could not be corrected for maternal weight.

In conclusion, our study demonstrates that low-dose antenatal prednisone exposure and maternal RA disease activity during pregnancy are not associated with long-term elevated cortisol and cortisone concentrations, an altered body composition, or higher blood pressure in prepubertal childhood. Prednisone is an effective, low-priced antirheumatic drug that is considered safe to use during pregnancy. The findings of this study support the assumption that maternal prednisone use during pregnancy is safe for the offspring, at least until the age of approximately 10 years, which is reassuring for mothers who use prednisone and their physicians.

REFERENCES

1. Levitt NS, Lindsay RS, Holmes MC, Seckl JR. Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. Neuroendocrinology 1996;64:412-8.
2. Cottrell EC, Seckl JR. Prenatal stress, glucocorticoids and the programming of adult disease. Front Behav Neurosci 2009;3:19.
3. Singh RR, Cuffe JS, Moritz KM. Short- and long-term effects of exposure to natural and synthetic glucocorticoids during development. Clin Exp Pharmacol Physiol 2012;39:979-89.
4. Seckl JR. Prenatal glucocorticoids and long-term programming. Eur J Endocrinol 2004;151 Suppl 3:U49-62.
5. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, et al. A transgenic model of visceral obesity and the metabolic syndrome. Science 2001;294:2166-70.
6. Desai M, Jellyman JK, Han G, Beall M, Lane RH, Ross MG. Maternal obesity and high-fat diet program offspring metabolic syndrome. American journal of obstetrics and gynecology 2014;211:237.e1-.e13.
7. Zambrano E, Nathanielsz PW. Mechanisms by which maternal obesity programs offspring for obesity: evidence from animal studies. Nutr Rev 2013;71 Suppl 1:S42-54.
8. O'Connor TG, Bergman K, Sarkar P, Glover V. Prenatal cortisol exposure predicts infant cortisol response to acute stress. Dev Psychobiol 2013;55:145-55.
9. Diego MA, Field T, Hernandez-Reif M, Cullen C, Schanberg S, Kuhn C. Prepartum, postpartum, and chronic depression effects on newborns. Psychiatry 2004;67:63-80.
10. Brennan PA, Pargas R, Walker EF, Green P, Newport DJ, Stowe Z. Maternal depression and infant cortisol: influences of timing, comorbidity and treatment. J Child Psychol Psychiatry 2008;49:1099-107.
11. Osborne S, Biaggi A, Chua TE, Du Preez A, Hazelgrove K, Nikkheslat N, et al. Antenatal depression programs cortisol stress reactivity in offspring through increased maternal inflammation and cortisol in pregnancy: The Psychiatry Research and Motherhood - Depression (PRAM-D) Study. Psychoneuroendocrinology 2018;in press.
12. Grant KA, McMahon C, Austin MP, Reilly N, Leader L, Ali S. Maternal prenatal anxiety, postnatal caregiving and infants' cortisol responses to the still-face procedure. Dev Psychobiol 2009;51:625-37.
13. Gutteling BM, de Weerth C, Buitelaar JK. Prenatal stress and children's cortisol reaction to the first day of school. Psychoneuroendocrinology 2005;30:541-9.
14. O'Connor TG, Ben-Shlomo Y, Heron J, Golding J, Adams D, Glover V. Prenatal anxiety predicts individual differences in cortisol in pre-adolescent children. Biol Psychiatry 2005;58:211-7.
15. Bjorntorp P, Rosmond R. Obesity and cortisol. Nutrition 2000;16:924-36.
16. Kelly JJ, Mangos G, Williamson PM, Whitworth JA. Cortisol and hypertension. Clin Exp Pharmacol Physiol Suppl 1998;25:S51-6.
17. Gillman MW, Rich-Edwards JW, Huh S, Majzoub JA, Oken E, Taveras EM, et al. Maternal corticotropin-releasing hormone levels during pregnancy and offspring adiposity. Obesity (Silver Spring, Md) 2006;14:1647-53.
18. Rondo PH, Lemos JO, Pereira JA, Souza JM. The relationship between cortisol concentrations in pregnancy and systemic vascular resistance in childhood. Early Hum Dev 2010;86:127-31.
19. Sen S, Carpenter AH, Hochstadt J, Huddleston JY, Kustanovich V, Reynolds AA, et al. Nutrition, weight gain and eating behavior in pregnancy: a review of experimental evidence for long-term effects on the risk of obesity in offspring. Physiol Behav 2012;107:138-45.
20. Aubuchon-Endsley NL, Bublitz MH, Stroud LR. Pre-pregnancy obesity and maternal circadian cortisol regulation: Moderation by gestational weight gain. Biological psychology 2014;102:38-43.
21. D'Anna-Hernandez KL, Ross RG, Natvig CL, Laudenslager ML. Hair cortisol levels as a retrospective marker of hypothalamic-pituitary axis activity throughout pregnancy: comparison to salivary cortisol. Physiol Behav 2011;104:348-53.
22. Benediktsson R, Calder AA, Edwards CR, Seckl JR. Placental 11 beta-hydroxysteroid dehydrogenase: a key regulator of fetal glucocorticoid exposure. Clin Endocrinol (Oxf) 1997;46:161-6.

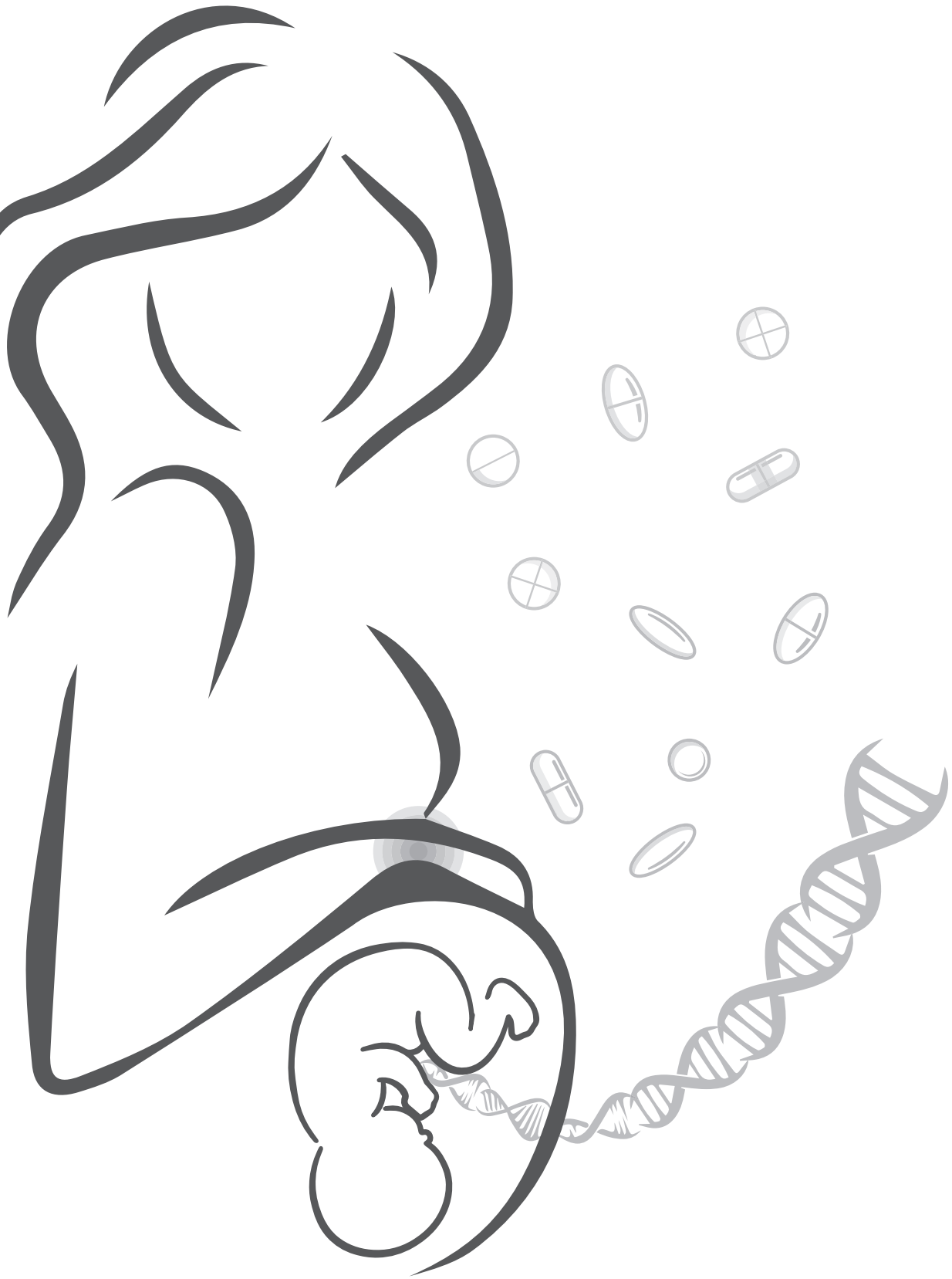
23. Kieviet N, de Groot S, Noppe G, de Rijke YB, van Rossum EF, van den Akker EL, et al. Is poor neonatal adaptation after exposure to antidepressant medication related to fetal cortisol levels? An explorative study. Early Hum Dev 2016;98:37-43.
24. Rippe RC, Noppe G, Windhorst DA, Tiemeier H, van Rossum EF, Jaddoe VW, et al. Splitting hair for cortisol? Associations of socio-economic status, ethnicity, hair color, gender and other child characteristics with hair cortisol and cortisone. Psychoneuroendocrinology 2016;66:56-64.
25. Burton PJ, Waddell BJ. Dual function of 11beta-hydroxysteroid dehydrogenase in placenta: modulating placental glucocorticoid passage and local steroid action. Biol Reprod 1999;60:234-40.
26. de Steenwinkel FD, Hokken-Koelega AC, Hazes JM, Dolhain RJ. The influence of foetal prednisone exposure on the cortisol levels in the offspring. Clin Endocrinol (Oxf) 2014;80:804-10.
27. Edwards CR, Benediktsson R, Lindsay RS, Seckl JR. Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? Lancet 1993;341:355-7.
28. Kossintseva I, Wong S, Johnstone E, Guilbert L, Olson DM, Mitchell BF. Proinflammatory cytokines inhibit human placental 11beta-hydroxysteroid dehydrogenase type 2 activity through Ca2+ and cAMP pathways. Am J Physiol Endocrinol Metab 2006;290:E282-8.
29. Staufenbiel SM, Penninx BW, Spijker AT, Elzinga BM, van Rossum EF. Hair cortisol, stress exposure, and mental health in humans: a systematic review. Psychoneuroendocrinology 2013;38:1220-35.
30. Veldhorst MA, Noppe G, Jongejan MH, Kok CB, Mekic S, Koper JW, et al. Increased scalp hair cortisol concentrations in obese children. J Clin Endocrinol Metab 2014;99:285-90.
31. Russell E, Koren G, Rieder M, Van Uum S. Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. Psychoneuroendocrinology 2012;37:589-601.
32. Short SJ, Stalder T, Marceau K, Entringer S, Moog NK, Shirtcliff EA, et al. Correspondence between hair cortisol concentrations and 30-day integrated daily salivary and weekly urinary cortisol measures. Psychoneuroendocrinology 2016;71:12-8.
33. Gow R, Thomson S, Rieder M, Van Uum S, Koren G. An assessment of cortisol analysis in hair and its clinical applications. Forensic Sci Int 2010;196:32-7.
34. Sauve B, Koren G, Walsh G, Tokmakejian S, Van Uum SH. Measurement of cortisol in human hair as a biomarker of systemic exposure. Clin Invest Med 2007;30:E183-91.
35. Xie Q, Gao W, Li J, Qiao T, Jin J, Deng H, et al. Correlation of cortisol in 1-cm hair segment with salivary cortisol in human: hair cortisol as an endogenous biomarker. Clin Chem Lab Med 2011;49:2013-9.
36. Noppe G, Van Rossum EF, Koper JW, Manenschijn L, Bruining GJ, de Rijke YB, et al. Validation and reference ranges of hair cortisol measurement in healthy children. Horm Res Paediatr 2014;82:97-102.
37. Groeneveld MG, Vermeer HJ, Linting M, Noppe G, van Rossum EF, van IMH. Children's hair cortisol as a biomarker of stress at school entry. Stress 2013;16:711-5.
38. Noppe G, van den Akker EL, de Rijke YB, Koper JW, Jaddoe VW, van Rossum EF. Long-term glucocorticoid concentrations as a risk factor for childhood obesity and adverse body-fat distribution. Int J Obes (Lond) 2016;40:1503-9.
39. de Man YA, Dolhain RJ, van de Geijn FE, Willemsen SP, Hazes JM. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum 2008;59:1241-8.
40. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
41. Smit MP, van Leer EHG, Noppe G, de Rijke YB, Kramer van Driel D, van den Akker ELT. Long-Term Cortisol Concentration in Scalp Hair of Asthmatic Children Using Inhaled Corticosteroids: A Case-Control Study. Hormone Research in Pædiatrics 2017;88:231-6.
42. Manenschijn L, Koper JW, Lamberts SW, van Rossum EF. Evaluation of a method to measure long term cortisol levels. Steroids 2011;76:1032-6.
43. Dettenborn L, Tietze A, Kirschbaum C, Stalder T. The assessment of cortisol in human hair: associations with sociodemographic variables and potential confounders. Stress 2012;15:578-88.
44. Noppe G, de Rijke YB, Dorst K, van den Akker EL, van Rossum EF. LC-MS/MS-based method for long-term steroid profiling in human scalp hair. Clin Endocrinol (Oxf) 2015;83:162-6.
45. Pragst F, Balikova MA. State of the art in hair analysis for detection of drug and alcohol abuse. Clin Chim Acta 2006;370:17-49.

46. Karlen J, Frostell A, Theodorsson E, Faresjo T, Ludvigsson J. Maternal influence on child HPA axis: a prospective study of cortisol levels in hair. Pediatrics 2013;132:e1333-40.
47. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77-97.
48. Galescu O, George M, Basetty S, Predescu I, Mongia A, Ten S, et al. Blood Pressure over Height Ratios: Simple and Accurate Method of Detecting Elevated Blood Pressure in Children. Int J Pediatr 2012;2012:253497.
49. Talma H., Bakker B., HiraSing R.A., S. vB. Groeidiagrammen 2010. Handleiding bij het meten en wegen van kinderen en het invullen van groeidiagrammen. . TNO Kwaliteit van Leven, Leiden 2010;ISBN:978-90-5986-347-7.
50. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). Acta Paediatr Scand 1991;80:756-62.
51. Staufenbiel SM, Penninx BW, de Rijke YB, van den Akker EL, van Rossum EF. Determinants of hair cortisol and hair cortisone concentrations in adults. Psychoneuroendocrinology 2015;60:182-94.
52. de Steenwinkel FDO, Dolhain R, Hazes JMW, Hokken-Koelega ACS. Does prednisone use or disease activity in pregnant women with rheumatoid arthritis influence the body composition of their offspring? Reprod Toxicol 2017;71:118-23.
53. Tengstrand B, Hafstrom I. Bone mineral density in men with rheumatoid arthritis is associated with erosive disease and sulfasalazine treatment but not with sex hormones. J Rheumatol 2002;29:2299-305.
54. Majumdar SR, Morin SN, Lix LM, Leslie WD. Influence of recency and duration of glucocorticoid use on bone mineral density and risk of fractures: population-based cohort study. Osteoporos Int 2013;24:2493-8.
55. de Steenwinkel FD, Hokken-Koelega AC, Hazes JM, Dolhain RJ. Brief report: does medication use or disease activity during pregnancy in patients with rheumatoid arthritis affect bone density in their prepubertal offspring? Arthritis Rheumatol 2014;66:533-7.
56. Gotestam Skorpen C, Hoeltzenbein M, Tincani A, Fischer-Betz R, Elefant E, Chambers C, et al. The EULAR points to consider for use of antirheumatic drugs before pregnancy, and during pregnancy and lactation. Ann Rheum Dis 2016;75:795-810.

Supplemental table 1. Detailed descriptive statistics of study population

	Prednisone (N=56)	Non-prednisone (N=61)	Reference population (n=150)
Weight kg, mean (sd)	36.7 (12.5)	35.6 (12.0)	36.5 (14.1)
Weight sds, mean (sd)	0.4 (1.0)	0.5 (1.1)	0.3 (0.9)
Height cm, mean (sd)	143.2 (16.7)	142.2 (16.0)	143.6 (19.5)
Height sds, mean (sd)	-0.1 (1.0)	0.1 (1.1)	0.3 (0.8)*
BMI child, mean (sd)	17.4 (2.3)	17.1 (2.7)	16.9 (2.6)
BMI child sds, mean (sd)	0.4 (0.9)	0.3 (1.2)	0.0 (1.1)*
Overweight	7 (12.5)	7 (11.5)	14 (9.3)
Systolic BP mmHg, mean (sd)	107.2 (11.5)	106.0 (11.2)	-
Ratio systolic BP/height, mean (sd)	0.8 (0.1)	0.7 (0.1)	-
Diastolic BP mmHg, mean (sd)	63.5 (6.8)	65.8 (8.5)	-
Ratio systolic BP/height, median (sd)	0.4 (0.1)	0.5 (0.1)	-
Waist circumference cm, mean (sd)	59.0 (6.8)	58.0 (8.2)	64.4 (9.2)**
Waist circumference sds, mean (sd)	-0.3 (1.1)	-0.5 (1.3)	0.8 (0.8)**
Hip circumference cm, mean (sd)	69.3 (9.9)	67.9 (9.6)	73.0 (10.3)**
Hip circumference sds, mean (sd)	-0.4 (1.0)	-0.5 (1.1)	0.3 (1.1)**
Waist-hip ratio, mean (sd)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)
Fat percentage, mean (sd)	18.5 (5.7)	19.2 (5.7)	-
Fat percentage sds, mean (sd)	-0.1 (1.1)	0.0 (1.1)	-
Skinfolds, mm			-
Biceps, mean (sd)	6.2 (2.9)	7.0 (3.9)	
Triceps, mean (sd)	9.4 (3.4)	9.6 (4.0)	
Subscapulair, median (IQR)	6.0 (4.3 – 7.3)	5.7 (5.0 – 7.7)	
Suprailiac, median (IQR)	6.6 (5.0 – 9.3)	6.3 (4.7 – 10.0)	
Skinfolds sds			
Biceps, mean (sd)	0.4 (1.5)	0.8 (2.3)	
Triceps, mean (sd)	0.2 (1.2)	0.3 (1.6)	
Subscapulair, median (IQR)	0.1 (-1.1 – 0.9)	0.0 (-0.7 – 0.7)	
Suprailiac, median (IQR)	-0.9 (-1.6 - -0.2)	-1.0 (-1.6 – 0.1)	
Ratio skinfolds trunk to peripheral, mean (sd)	0.9 (0.3)	1.0 (0.4)	-

* p-values <0.05 **p-values <0.001, statistics were performed comparing the prednisone and non-prednisone group, and the prednisone and non-prednisone group together versus the reference population.
sd=standard deviation; sds=standard deviation scores; IQR=interquartile range; SES=socioeconomic status; BP=blood pressure; DAS28-CRP (3)=disease activity score in 28 joints using C-reactive protein levels



In rheumatoid arthritis fewer women breastfeed their offspring compared with women from the general population; results from a nationwide prospective cohort study

Hilal Ince-Aşkan
Johanna M.W. Hazes
Radboud J.E.M. Dolhain

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ABSTRACT

Objectives. The World Health Organization recommends to exclusively breastfeed infants until the age of six months. The first objective was to compare breastfeeding frequencies and time of cessation between women with rheumatoid arthritis (RA) and the general population. Second, to identify why patients with RA discontinue breastfeeding.

Methods. This study was embedded in the Pregnancy induced Amelioration of Rheumatoid Arthritis study, a nationwide prospective cohort study. From 2002–2008 in total 249 pregnancies were followed from pregnancy until 6 months postpartum. Data on lactation and medication use were collected. Proportion tests were used to compare percentages of breastfeeding between the study population and the general/reference population.

Results. At 4-6, 12 and 26 weeks postpartum 43%, 26% and 9% of the patients with RA breastfed their offsprings respectively, compared with 63%, 46% and 41% in the general population (p-values<0.001). The main reason for women to discontinue breastfeeding was the restart of medication (n=129, 58.6%). Nevertheless, more than 40% of these patients restarted medication that was considered compatible with breastfeeding.

Conclusions. This large prospective study demonstrates that RA is associated with lower proportions of women breastfeeding their offspring and earlier cessation compared with the general population. A considerable amount of patients discontinue breastfeeding so that they could start medication, despite the fact that many of the medications are considered safe to use during lactation. Using the results of this study, intervention strategies supporting patients with RA who wish to breastfeed may be developed.

Research on the impact of pregnancy on rheumatoid arthritis (RA) and the consequences of RA on pregnancy has gained more and more interest in the last years. However, breastfeeding in patients with RA is a neglected area, since there are limited numbers of studies on this subject. There are no studies available that compare breastfeeding frequency and duration in RA with the general population Considering lactation in patients with RA, one prospective study, that focused on disease activity in relation with breastfeeding, showed worse disease activity 6 months postpartum in first-time breastfeeding women compared with non-breastfeeding women[1].

The World Health Organization (WHO) recommends to exclusively breastfeed infants until the age of six months, and to continue breastfeeding until the age of two years (or beyond) alongside complementary foods[2]. Besides being the most optimal infant nutrition and improving the bond between mother and child[3, 4], breast milk also has other long-term benefits[2]. Breastfed children have lower risk of infectious diseases[5], sudden infant death syndrome[6], asthma[7, 8] obesity, high cholesterol or high blood pressure later in life[9, 10]. Breastfeeding especially has major benefits for high-risk infants born prematurely[5]. Since RA may have a negative impact on pregnancy outcome[11], breastfeeding might be utmost important for their offsprings.

In view of the increased risk of a flare of RA within 3 months postpartum, medication is often restarted after delivery[12]. Safety data on the use of most antirheumatic drugs during lactation are absent or limited[13]. At the time this study was conducted, medication that was considered safe during lactation included conventional synthetic Disease Modifying AntiRheumatic Drugs (DMARDs) like hydroxychloroquine, sulfasalazine, and anti-inflammatory drugs like prednisone and non-selective NSAIDs[14]. Drugs that were advised to be avoided during lactation included methotrexate (MTX) and leflunomide. Biologicals, all selective COX II inhibitors and azathioprine were also not recommended to be prescribed during lactation (at the time this study was conducted)[14].

The aims of the current prospective study were firstly to compare the frequency and duration of breastfeeding in women with RA with the general population. Secondly, to identify reasons why patients with RA discontinue breastfeeding. Finally, to identify clinical factors in the 3rd trimester during pregnancy associated with discontinuation of breastfeeding within three months postpartum. This study will help us to understand the impact of RA on lactation, and to develop intervention strategies to support patients with RA who wish to breastfeed.

PATIENTS AND METHODS

Study Population

The study cohort consisted of women who participated in the Pregnancy induced Amelioration of Rheumatoid Arthritis (PARA) study, a nationwide prospective cohort study from the Netherlands with an inclusion period from 2002 to 2008 (last visit in 2010), described in detail previously[15]. Patients were recruited by their rheumatologist if they met the American College of Rheumatology 1987 revised criteria for RA[16]. Inclusion was possible if patients had a wish to conceive, or when they were already pregnant, preferably in the first trimester. In total, 249 pregnancies were available for the current analysis[17].

The reference group consists of 3009 women recruited throughout the Netherlands in 2005 for a population-based study on breastfeeding and infant formula, representative of the general population at that time[18]. Women were eligible for inclusion if their infant was aged 6 months or younger.

Data collection

In the PARA-study, patients were visited by a research nurse at home preferably before pregnancy, three times during pregnancy and three times after the birth of the child (6, 12 and 26 weeks). At first visit, data on demographics was collected. At all time-points a physical examination was performed and information on mother (e.g. disease activity, functionality, presence of erosions, medication) and child was collected. At the postpartum visits, data on child feeding was collected using questionnaires. These included questions on the start and duration of breastfeeding, on exclusive breastfeeding or combined with infant formula, or exclusively infant formula, and on the reason for discontinuing breastfeeding (restart of medication, maternal reasons, child related reasons, or a combination). Maternal reason were: no desire to, mastitis, work, too much effort, not enough milk, breast surgery. Child related reasons were: struggle to latch, failure to thrive, illness.

The Disease Activity Score based upon 3 variables; swelling and tenderness by palpation in 28 joints and a C-Reactive Protein (DAS28-CRP(3)) was used to measure RA disease activity[19, 20]. Remission was defined as a DAS28-CRP(3) <2.6, low disease activity as ≥2.6 to <3.2, intermediate disease activity as ≥3.2 to ≤5.1, and high disease activity as >5.1[15]. In 10 patients, the DAS28-CRP(3) from the third trimester was missing. Since the correlation between the DAS28-CRP(3) between second and third trimester was high (0.7), the DAS28-CRP(3) was substituted with the DAS28-CRP(3) from the second trimester in these 10 patients.

As a measure for functionality, the conventional health assessment questionnaire (HAQ) score was determined using the validated Dutch translation of the Stanford HAQ, which considers the use of devices and aids[20-22]. Rheumatoid Factor (RF) and anti-citrullinated protein antibody (ACPA) were measured in the 3rd trimester.

In the reference group questionnaires were used to determine among others frequency and duration of breastfeeding, frequency of exclusive breastfeeding or combined with infant formula, or exclusively infant formula. In addition, data on demographics, and labor and postnatal care was collected.

Statistical Analysis

Descriptive statistics were calculated as numbers, percentages, means, medians, standard deviation scores (SDSs) and interquartile ranges (IQRs). Proportion tests were used to compare percentages of breastfeeding between the study population and the general/reference population. Chi squared tests were used to compare frequencies between groups. Student's *t*-tests were used to compare DAS28-CRP(3) between breastfeeding and non-breastfeeding patients.

Subgroups based on birthweight were created (birthweight <2500g and ≥2500g) in children born to women who participated in the PARA-study. Chi squared tests were used to compare breastfeeding frequencies between low and normal birthweight infants.

A multivariable logistic regression model was built for the analysis of the association of clinical factors in the 3rd trimester with cessation of breastfeeding within 12 weeks. Covariables with a p-value<0.2 in the univariable analysis were used in the multivariable model. After that, the model was fitted step-by-step using backwards selection of variables with p-values>0.2. The dependent variable was discontinuation of breastfeeding before 12 weeks postpartum. Independent variables were the HAQ in the 3rd trimester, the autoantibody status, use of MTX in the past, the socioeconomic status (SES) based on education level, prednisone use in the 3rd trimester, sulfasalazine use in the 3rd trimester, the presence of erosions, the DAS28-CRP(3) in the 3rd trimester, maternal smoking periconceptional and/or during pregnancy, the visual analogue scale for global health (VAS GH) in the 3rd trimester, maternal age and parity.

A subgroup analysis was performed including only the first participation of the patients in the PARA-study. Breastfeeding frequencies were once again analyzed using Chi squares tests, and the regression model was repeated in this group.

Statistical significance was defined as p ≤0.05. All statistics were performed using STATA software version 15.1 for Windows.

Ethics

This study is in compliance with the Helsinki Declaration. The Medical Ethics Committee at the Erasmus Medical Center Rotterdam, the Netherlands, approved the PARA-study (MEC-214.320/2002/117). All participants provided written informed consent.

RESULTS

Participants

In the PARA-study, in total 369 patients were included, which resulted in 256 successful pregnancies. The DAS28-CRP(3) in the second and third trimester was missing in 7 pregnancies. After exclusion of these, in total data on 249 pregnancies from 216 women was available for the current analysis. Descriptive statistics of the study population are shown in table 1. The mean maternal age at delivery was 32.8 years and the median duration of RA was 4.9 years. The mean DAS28-CRP(3) was 3.4 in the third trimester. In total, 134 patients (53.8%) had a DAS28-CRP(3) ≥3.2 (intermediate to high disease activity) in the third trimester.

Since the median HAQ in the third trimester was 0.75 (IQR= 0.25 – 1.25), the HAQ was dichotomized to a lower (<0.75) and higher (≥0.75) HAQ. This resulted in 114 patients (45.8%) with a lower, and 135 patients (54.2%) with a higher HAQ.

In total, 186 (74.7%) were classified as RF and/or ACPA positive. Erosions were present in 150 patients (60.2%). Approximately half of the patients had a high SES based on educational level (university of applied sciences and academic university education). In total 22 patients (8.8%) smoked periconceptional or during pregnancy.

Breastfeeding during pregnancy

In total (exclusive and partial breastfeeding combined), 108 patients (43%) breastfed their offspring until at least 4-6 weeks, 65 (26%) until at least 12 weeks and 23 (9%) until at least 26 weeks postpartum compared with 63%, 46%, and 41% respectively in the reference population (all p-values<0.001) (Table 2). In our study, breastfeeding frequency and duration was not different between nulliparous and multiparous patients with RA.

Since breastmilk especially has benefits for infants with a low birth weight[5], subgroups based on birthweight were created in the offsprings born to women with RA from the PARA-study, resulting in 23 infants with a birthweight <2500g and 226 ≥2500g. Only 26.1%, 17.4% and 4.4% of the offsprings <2500g were breastfed at 4-6 weeks, 12 weeks and 26 weeks respectively compared with 45.1%, 27.0% and 9.7% respectively in the offsprings with a birthweight ≥2500g (not significant).

In total, 33 women participated more than once in this study. To exclude possible selection bias in the obtained results, a subgroup analysis, including only the first participation of the patients (n=216) was performed. Similar results in breastfeeding frequency and duration were obtained (data not shown).

Table 1. Descriptive statistics of study population.*

	Baseline characteristics (n=249 pregnancies)
Maternal age at delivery, mean (sd) years	32.8 (3.8)
Duration RA at baseline, median (IQR) years	4.9 (2.2 – 9.8)
DAS28 3rd trimester, mean (sd)	3.4 (1.1)
3rd trimester DAS28-CRP#	
<2.6	72 (28.9)
≥2.6 to <3.2	43 (17.2)
≥3.2 to ≤5.1	118 (47.4)
>5.1	16 (6.4)
HAQ in 3rd trimester, median (IQR)	0.75 (0.25 – 1.25)
Parity	
Nulliparous (no previous offsprings)	124 (51.5)
Multiparous (≥1 previous offspring)	117 (48.5)
RA-associated autoantibodies	
Either RF or ACPA positive, or both positive	186 (74.7)
Both negative	63 (25.3)
RF positive	172 (69.1)
ACPA positive	153 (61.5)
Presence of erosions	150 (60.2)
Methotrexate use in the past	143 (57.4)
Biological agent use in the past	44 (17.7)
Medication use during 3rd trimester	
Prednisone only	63 (25.3)
Sulfasalazine only	37 (14.9)
Both prednisone and sulfasalazine	22 (8.8)
Hydroxychloroquine (either alone or in combination)	4 (1.6)
No medication during 3rd trimester	123 (49.4)
SES based on educational level	
Low	16 (6.4)
Middle	79 (31.7)
High	131 (52.6)
Unknown	23 (9.2)
Smoking$	22 (8.8)

*Values are number (%) unless indicated otherwise. Normally distributed data is presented with mean (sd), not normally distributed data is presented with median (IQR). RA= rheumatoid arthritis; IQR= interquartile range; RF= rheumatoid factor; ACPA= anti-citrullinated protein antibody; DAS28-CRP(3)= disease activity score in 28 joints using C-reactive protein levels.
Disease activity groups are defined according to the European League Against Rheumatism criteria. $ Smoking periconceptional or during pregnancy

Table 2. Numbers and percentages of breastfeeding women 4-6 weeks, 12 weeks and 26 weeks postpartum in the PARA-study and in the general population (Netherlands, 2005 [18])*.

	RA, PARA-study (n=249)			General population (n=3009)		
	Total	Exclusive	Partial	Total	Exclusive	Partial
4-6 weeks	108 (43)#	91 (36)	17 (7)	1896 (63)#	1625 (54)	271 (9)
12 weeks	65 (26)#	47 (19)	18 (7)	1384 (46)#	1053 (35)	331 (11)
26 weeks	23 (9)#	11 (4)	12 (5)	1233 (41)#	752 (25)	481 (16)

*Values are number (%) unless indicated otherwise. # p-value <0.001 between RA and general population
RA= rheumatoid arthritis

Table 3. Reasons for discontinuation of breastfeeding reported by the patients*.

	Discontinuation < 6 weeks postpartum (n=141)	Discontinuation 6 - 12 weeks postpartum (n=43)	Discontinuation 12 - 26 weeks postpartum (n=39)	Total (n=223)
Restart of medication				
Total	86 (61.0)	28 (65.1)	15 (38.5)	129 (57.8)
Not compatible	51	17	8	76
Compatible	35	11	7	53
Maternal reasons	44 (31.2)	14 (32.6)	19 (48.7)	77 (34.5)
No desire to	15	.	1	16
Not enough milk	13	8	12	33
Work related	.	4	3	7
Too much effort	7	.	2	9
Mastitis	4	1	.	5
Breast surgery	3	.	.	3
Unknown	.	1	1	2
Child related reasons	11 (7.8)	1 (2.3)	3 (7.7)	15 (6.7)
Struggle to latch	6	.	1	7
Failure to thrive	4	1	1	6
Illness	1	.	1	2
Combination of maternal and child related reasons	.	.	1 (2.6)	1 (0.4)
Unknown	.	.	1	1
Unknown	.	.	1 (2.6)	1 (0.4)

*Values are number (%) unless indicated otherwise.

Reasons for discontinuation of breastfeeding

In total, 223 patients discontinued breastfeeding over the course of 26 weeks. In 129 patients (58.6%), the reason for discontinuation of breastfeeding was the restart of medication (Table 3). From these 129 patients, in total 86 patients (61.0%) reported that the reason for cessation was the restart of medication before 6 weeks, 28 patients (65.1%) between 6-12 weeks and 15 patients (41.7%) between 12-26 weeks postpartum. In 76/129 patients (58.9%) medication that was considered not compatible with lactation (at that time this study was conducted) was initiated. The other 53 patients (41.1%) received (a combination of) prednisone (49.1%), sulfasalazine (39.6%), hydroxychloroquine (18.9%), and/or non-selective NSAIDs (37.7%), all considered safe to use during lactation.

In total, 94 patients (42.2%) reported reasons for cessation, other than the restart of medication. From these, the majority (n=77, 81.9%) included maternal reasons, e.g. "not enough milk" (35.1%) and "no desire to" (17.0%). As for child related reasons (n=15, 16.0%), the majority included "struggle to latch" (7.4%) and "failure to thrive" (6.4%), (see Table 3). In the general population (Netherlands 2000-2003[23]), cessation due to "not enough milk" (including concern about the amount of milk), "problems relating to breasts and/or nipples", and "health problems in the infant" were comparable with the results from this study. Work related cessation was reported in 7.4% of the patients in this study (in patients with reasons other than the restart of medication) compared with 13.9% in the general population (p-value<0.05)[23].

As for the subgroups based on birth weight, approximately half of the patients with an offspring <2500g reported discontinuation due to maternal reasons and the other half due to restart of medication. These patients received MTX or a combination (54.5%), sulfasalazine or a combination (27.3%), prednisone or a combination (63.6%), hydroxychloroquine or a combination (18.2%), and non-selective NSAIDs or a combination (27.3%).

Disease activity and medication use postpartum in breastfeeding and non-breastfeeding patients

The DAS28-CRP(3) changed from 3.1 to 3.4 to 3.0 at 6, 12, and 26 weeks after delivery in breastfeeding and from 3.5 to 3.7 to 3.5 in non-breastfeeding patients (p value at all time points between the two groups < 0.05).

Medication use postpartum is shown in table 4. Overall, patients who were not breastfeeding received more medication compared with patients who were breastfeeding. MTX and leflunomide were exclusively prescribed to non-breastfeeding patients.

Table 4. Disease activity and medication use postpartum*.

	DAS28-CRP(3)	Prednisone	SSZ	HCQ	Non- selective NSAIDs	MTX#	Leflunomide#	TNF inhibitors#	Selective COX II inhibitors#	Azathioprine#
Breastfeeding										
6 weeks (n=108)	3.1	21 (19.4)	23 (21.3)	0 (0)	4 (3.7)	0 (0)	0 (0)	1 (0.9)	0 (0)	3 (2.8)
12 weeks (n=65)	3.4	15 (23.1)	17 (26.2)	0 (0)	9 (13.8)	0 (0)	0 (0)	1 (1.5)	1 (1.5)	3 (4,6)
26 weeks (n=23)	3.0	3 (13.0)	7 (30.4)	0 (0)	4 (17.4)	0 (0)	0 (0)	0 (0)	0 (0)	3 (13.0)
Non-breastfeeding										
6 weeks (n=141)	3.5	65 (46.1)	42 (29.8)	10 (7.1)	37 (26.2)	46 (32.6)	0 (0)	12 (8.5)	8 (5.7)	3 (2.1)
12 weeks (n=184)	3.7	75 (40.8)	57 (31.0)	19 (10.3)	60 (32.6)	76 (41.3)	3 (1.6)	23 (12.5)	15 (8.2)	3 (1.6)
26 weeks (n=220)	3.5	75 (34.1)	63 (28.6)	18 (8.2)	64 (29.1)	92 (41.8)	4 (1.8)	29 (13.2)	16 (7.3)	2 (0.9)

* Values are number (%) unless indicated otherwise. # considered not compatible with lactation at the time this study was conducted SSZ= sulfasalazine; HCQ= hydroxychloroquine; NSAIDs= Nonsteroidal anti-inflammatory drugs; MTX= methotrexate; TNF= tumor necrosis factor

Factors in 3rd trimester associated with discontinuation of breastfeeding within 12 weeks

For clinical purposes the HAQ in the third trimester was dichotomized at the median for easier interpretation (<0.75 and ≥0.75). In the univariable analyses, the HAQ, the presence of autoantibodies, smoking periconceptional or during pregnancy, MTX use in the past, SES based on educational level, prednisone use in the third trimester, the presence of erosions and the DAS28-CRP(3) in the third trimester were associated with discontinuation within12 weeks (p-values<0.20) (Table 5).

In the multivariable model, the HAQ in the 3rd trimester, the presence of autoantibodies and smoking were statistically significant (OR 3.7, 3.2, and 5.9 respectively, and p-values<0.001, 0.003, and 0.032 respectively). Patients with a higher HAQ, patients who were autoantibody positive and patients who smoked periconceptional or during pregnancy were more likely to discontinue breastfeeding within 12 weeks postpartum. A lower SES based on educational level, prednisone use in the third trimester, MTX use in the past, the presence of erosions, and the DAS28-CRP(3) in the third trimester were not significantly associated with discontinuation within 12 weeks in the multivariable model (see Table 5).

Patients with a HAQ ≥0.75 were more likely to restart MTX, leflunomide and biologicals at 6 or 12 weeks postpartum compared with patients with a HAQ <0.75 (p=0.001). In addition, in patients with a HAQ ≥0.75 the disease activity postpartum was higher compared with patients with a HAQ<0.75 (DAS28-CRP(3) 3.8 vs 3.3, p-value=0.001).

Also, patients who were autoantibody positive were more likely to restart MTX, leflunomide and biologicals at 6 or 12 weeks postpartum compared with patients who were autoantibody negative (p= 0.019). The autoantibody positive patients had higher disease activity postpartum compared with autoantibody negative patients (DAS28-CRP(3) 3.7 vs 3.2, p-value<0.001).

In the subgroup including only the first participation of the patients (n=216), similar results for the regression models were obtained (data not shown).

Table 5. 3rd trimester factors associated with discontinuation of breastfeeding within 12 weeks

	Discontinuation of breastfeeding within 12 weeks#			
	Univariable		Multivariable$ (n=226)	
	OR	P- value	OR	P- value
HAQ 3rd trimester ≥0.75	3.121+	<0.001+	3.669*	<0.001*
Autoantibody status positive	2.649+	0.002+	3.210*	0.003*
Smoking%	3.841+	0.075+	5.902*	0.032*
MTX use in past	1.857+	0.034+	1.659	0.143
SES based on educational level	0.696+	0.156+	0.661	0.150
Prednisone use 3rd trimester	3.378+	0.001+	1.924	0.114
Presence of erosions	1.693+	0.071+	-	-
DAS28-CRP(3) 3rd trimester	1.305+	0.052+	-	-
Sulfasalazine use 3rd trimester	0.991	0.980		
VAS GH	0.999	0.474		
Maternal age	0.989	0.774		
Parity ≥ 1	1.106	0.737		

OR= Odds Ratio; HAQ= Health Assessment Questionnaire; MTX= methotrexate; SES= socioeconomic status; DAS28-CRP(3)= Disease Activity Score in 28 joints using C-reactive protein levels ; VAS GH= Visual Analogue Score Global Health. # Based on logistic regression $ Multivariable model after forward selection (limit for inclusion: P < 0.20) and backward selection (limit for exclusion: P ≥ 0.20) % smoking periconceptional or during pregnancy, + Variables taken forward if p <0.20, * Statistically significant in the multivariable model

DISCUSSION

In this large nationwide prospective study, we have shown that women with RA are less likely to breastfeed their offsprings compared with women from the reference population. More than half (57%) of the patients discontinue breastfeeding before 6 weeks. At 12 weeks only 26% of the patients were still breastfeeding (exclusively and partially combined) compared with 46% in the general (reference) population. At 6 months postpartum the difference is extremely large (9% vs 41% respectively).

The majority of the patients reported that they had discontinued breastfeeding due to restart of medication. However, more than 40% of the patients ceased breastfeeding due to start of medication that was considered compatible with lactation, according to guidelines at the time this study was conducted. These drugs included prednisone, sulfasalazine, hydroxychloroquine, and/or non-selective NSAIDs. Cessation while using compatible medication was therefore not related to one specific antirheumatic drug. The only exception was hydroxychloroquine, which was prescribed to 4 women during pregnancy, and none during breastfeeding. However, due to the small numbers, a coincidence cannot

be ruled out. This cessation of breastfeeding while using compatible medication could therefore perhaps be due to a general fear for taking medication when breastfeeding, a lack of knowledge, or generic preferences of women to not take any medication whilst breastfeeding. Unfortunately, in this study it was not assessed whether patients themselves did not feel comfortable to breastfeed when taking medication or whether this was discouraged by their physician. Retrospectively collecting data on this subject was not possible, since our study was a nationwide prospective study, and patients were recruited by rheumatologists from different hospitals, all over the country. In addition, we did not have informed consent from the patients to contact their rheumatologist to gather information on this subject.

Other reasons for discontinuation included in maternal reasons, e.g. "not enough milk" and "no desire to", and child related reasons e.g. "struggle to latch" and "failure to thrive". Besides restart of medication, the reasons for cessation in this study were comparable with the general population[23]. Work related discontinuation of breastfeeding was reported in 7.7% of the patients in this study compared with 13.9% in the reference population (p-value=0.05). The most likely explanation for this difference is the lower proportion of paid employment in patients with RA[24, 25].

A higher HAQ in the third trimester, the presence of autoantibodies, and maternal smoking were significantly associated with discontinuation of breastfeeding within 12 weeks. It is conceivable that the HAQ and autoantibody status is related with the severity of the disease. In our study population, patients with a higher HAQ and patients who were autoantibody positive, had a higher disease activity postpartum and were more likely to restart MTX, leflunomide and biologicals at 6 or 12 weeks postpartum. It makes therefore sense to conclude that both a high HAQ as well as autoantibody positivity identify patients that have a higher burden of disease and therefore are more likely to require additional medication postpartum, prohibiting them from breastfeeding.

In addition, since the HAQ is a measure of functional status[26] it is likely that patients with a higher HAQ discontinue breastfeeding due to functional impairment, either due to the physical difficulties associated with breastfeeding, or due to requirement of strong antirheumatic medications.

Furthermore, maternal smoking was associated with early cessation of breastfeeding. Smoking was not related to medication use or disease activity postpartum. It is known that women with a lower SES are more likely to smoke during and after pregnancy, and less likely to breastfeed their offsprings[27-31]. Although breast milk loses many of its health promoting properties when the mother smokes during lactation, smoking mothers are advised not to discontinue breastfeeding[32]. Unfortunately, in our study the number of smoking mothers was too small to study associations with SES in subgroups. In addition, SES was included in the multivariable model, and maternal smoking was independently associated with

discontinuation of breastfeeding within 12 weeks, regardless of SES. Unfortunately, in our reference population, there was no data on smoking or SES, therefore we could not make a comparison with our reference group.

In addition, RA patients with offsprings with a low birth weight also require additional awareness. It has been shown earlier that preterm born infants with (very) low birth weight benefit the most from breastmilk[5]. In our study cohort, 85% of the mothers with an offspring with a birthweight <2500g discontinued breastfeeding <6 weeks postpartum, while the mean DAS28-CRP(3) postpartum was comparable in both birthweight-groups (data not shown). Approximately half of the patients with an offspring <2500g reported discontinuation due to the restart of medication. According to the guidelines at the time this study was performed, sulfasalazine was considered not compatible with lactation in ill, stressed or premature infants[13, 14]. However, in that respect, patients receiving prednisone, hydroxychloroquine and non-selective NSAIDs could have continued breastfeeding. Unfortunately, in our reference population, there was no data on low birthweight infants, therefore we could not compare our data with our reference group. Limited publications on breastfeeding in low birth weight infants are available. In the Netherlands (2000-2003), 50% of the infants with a birthweight ≤3000g were breastfed at 4 weeks postpartum[23], compared with 38% in our study population (p-value=0.06). Outside the Netherlands, the results were not consistent. Some studies report similar numbers of lactation in low birth weight infants[33, 34] compared with healthy infants, while others[35, 36] report lower numbers.

Another interesting finding from our study worth to mention is that, the DAS28-CRP(3) postpartum was significantly lower in breastfeeding patients compared with non-breastfeeding patients, despite taking less medication (when divided in breastfeeding and non-breastfeeding patients at every single time-point). This seems in contrast to the results of a previous study[1] that reported that first-time breastfeeding, but not breastfeeding in subsequent pregnancies, was associated with significantly more increase in RA disease activity postpartum compared with non-breast feeders. In that study, the change in disease activity from third trimester to 6 months after delivery was compared and breastfeeding was defined as lactating for at least 4 weeks. When a similar analysis was performed in our study, no such association between first time breastfeeding and an increase of RA disease activity could be observed (data not shown). That we could not reproduce the data of that study might also be related to the fact that that study was performed in a different time period, in which one was more reluctant to treat pregnant and lactating women. In addition disease activity was assessed in a different way in that study compared to our study. Although women with less severe disease are more likely to start and continue breastfeeding, the results of our study do not support the hypothesis that breastfeeding itself causes an increase in RA disease activity[1], e.g. by inducing high levels of prolactin, which is known to have pro-inflammatory properties.

The results of this study will help to develop intervention strategies to support patients with RA who wish to breastfeed. First of all, since a large proportion of patients with RA do not start or continue breastfeeding for taking medication that in fact is compatible, education on medication use during lactation may be valuable for these patients and their doctors. Not only rheumatologists, but also specialized nurses, midwives, and lactation consultants should be trained to increase their knowledge on this subject. In addition, access to up-to date medication resources for patients and their clinicians (e.g. evidence based online databases) are required.

Other patient groups that may benefit from targeted breastfeeding support include, patients with offsprings with a low birth weight, and patients who smoke. Furthermore, since functional impairment might influence the start and duration of breastfeeding, ergonomic recommendations should be provided especially to patients with a high HAQ.

In addition, the results of this study clearly show that a substantial number of patients do not breastfeed because they are in need of medication that is considered not compatible with breastfeeding. In this respect it is important to realize that guidelines on antirheumatic drug prescription during lactation are often not based on proven side-effects or toxicity for the offspring, but based on the lack of safety data of those drugs, especially on transfer of medication into breastmilk[13]. This holds true for many medications, including methotrexate, one of the "cornerstones" of RA-treatment. In this respect the authors want to advocate that more research into his particular field is undertaken. That such research, although difficult to perform, is feasible, is shown by a recent study on the lack of transfer of certolizumab into breast milk[37].

This study has some limitations. Firstly, it was conducted approximately 10-16 years ago, and in time antirheumatic medication prescriptions have changed. However, we identified that there is a large group of patients that discontinue breastfeeding unnecessarily while using compatible antirheumatic drugs. This might reflect a lack of knowledge on this subject. It is questionable if the knowledge of doctors and nurses on this specific subject, and education of patients in the clinical practice has improved. Nevertheless, it would be interesting to perform a similar study on breastfeeding by patients with RA in this era of biologic therapies.

Secondly, in total, 33 women participated more than once in this study. To exclude possible selection bias in the obtained results, we performed a subgroup analysis, including only the first participation of the patients (n=216), and found comparable results in breastfeeding frequency and in the regression model.

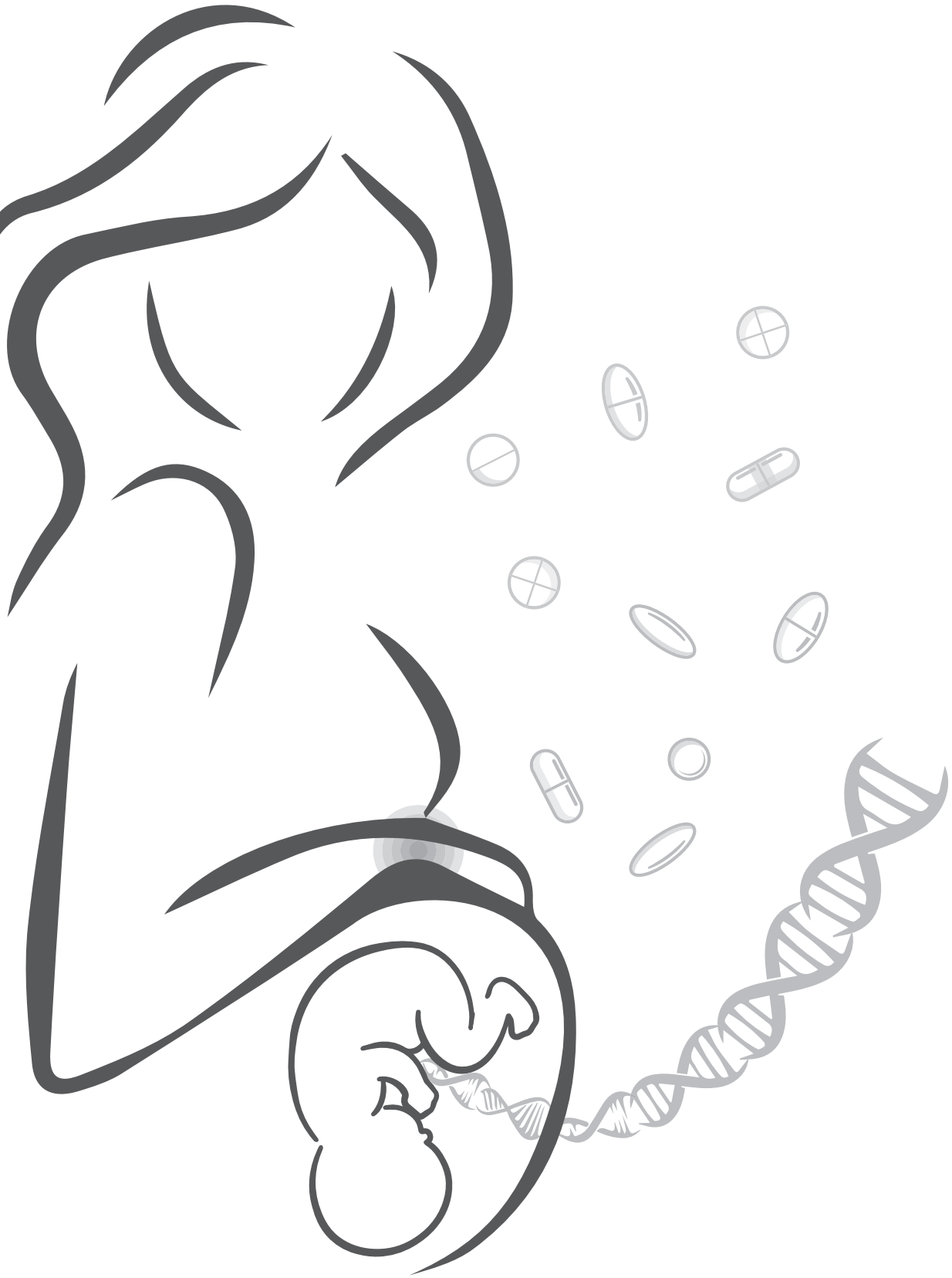
In conclusion, our study demonstrates that RA is associated with lower proportions of women breastfeeding their offspring and earlier cessation compared with the general population. A considerable amount of patients discontinue breastfeeding (unnecessarily) so that they could start medication, despite the fact that many of the medications are

considered safe to use during lactation. Given the known benefits of breastfeeding on the offspring, more effort in education in the clinical practice and more research into the transfer of medication into breastmilk might enable more patients with RA to breastfeed.

REFERENCES

1. Barrett JH, Brennan P, Fiddler M, Silman A. Breast-feeding and postpartum relapse in women with rheumatoid and inflammatory arthritis. Arthritis Rheum 2000;43:1010-5.
2. Daly A, Pollard CM, Phillips M, Binns CW. Benefits, barriers and enablers of breastfeeding: factor analysis of population perceptions in Western Australia. PLoS One 2014;9:e88204.
3. Liu J, Leung P, Yang A. Breastfeeding and active bonding protects against children's internalizing behavior problems. Nutrients 2013;6:76-89.
4. Anderson GC, Moore E, Hepworth J, Bergman N. Early skin-to-skin contact for mothers and their healthy newborn infants. Birth 2003;30:206-7.
5. Gharib S, Fletcher M, Tucker R, Vohr B, Lechner BE. Effect of Dedicated Lactation Support Services on Breastfeeding Outcomes in Extremely-Low-Birth-Weight Neonates. J Hum Lact 2017;in press:890334417741304.
6. Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, et al. Breastfeeding and maternal and infant health outcomes in developed countries. Evid Rep Technol Assess (Full Rep) 2007;1:1-186.
7. Oddy WH. Breast feeding and childhood asthma. Thorax 2009;64:558-9.
8. Oddy WH. The long-term effects of breastfeeding on asthma and atopic disease. Adv Exp Med Biol 2009;639:237-51.
9. Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. Pediatrics 2005;115:1367-77.
10. Owen CG, Whincup PH, Kaye SJ, Martin RM, Davey Smith G, Cook DG, et al. Does initial breastfeeding lead to lower blood cholesterol in adult life? A quantitative review of the evidence. Am J Clin Nutr 2008;88:305-14.
11. de Man YA, Hazes JM, van der Heide H, Willemsen SP, de Groot CJ, Steegers EA, et al. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: results of a national prospective study. Arthritis Rheum 2009;60:3196-206.
12. Makol A, Wright K, Amin S. Rheumatoid arthritis and pregnancy: safety considerations in pharmacological management. Drugs 2011;71:1973-87.
13. Gotestam Skorpen C, Hoeltzenbein M, Tincani A, Fischer-Betz R, Elefant E, Chambers C, et al. The EULAR points to consider for use of antirheumatic drugs before pregnancy, and during pregnancy and lactation. Ann Rheum Dis 2016;75:795-810.
14. Ostensen M, Khamashta M, Lockshin M, Parke A, Brucato A, Carp H, et al. Anti-inflammatory and immunosuppressive drugs and reproduction. Arthritis Res Ther 2006;8:209.
15. de Man YA, Dolhain RJ, van de Geijn FE, Willemsen SP, Hazes JM. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum 2008;59:1241-8.
16. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
17. Ince-Aşkan H, Hazes JMW, Dolhain R. Identifying Clinical Factors Associated With Low Disease Activity and Remission of Rheumatoid Arthritis During Pregnancy. Arthritis Care Res (Hoboken) 2017;69:1297-303.
18. Lanting C, Wouwe JV. Peiling melkvoeding van zuigelingen 2005: Borstvoeding in Nederland en relatie met certificering door stichting Zorg voor Borstvoeding (Breastfeeding in the Netherlands, 2005). TNO Kwaliteit van Leven, Leiden 2006;.1-18.
19. de Man YA, Dolhain RJ, Hazes JM. Disease activity or remission of rheumatoid arthritis before, during and following pregnancy. Curr Opin Rheumatol 2014;26:329-33.
20. de Man YA, Hazes JM, van de Geijn FE, Krommenhoek C, Dolhain RJ. Measuring disease activity and functionality during pregnancy in patients with rheumatoid arthritis. Arthritis Rheum 2007;57:716-22.
21. Bruce B, Fries JF. The Stanford Health Assessment Questionnaire: a review of its history, issues, progress, and documentation. J Rheumatol 2003;30:167-78.
22. Zandbelt MM, Welsing PM, van Gestel AM, van Riel PL. Health Assessment Questionnaire modifications: is standardisation needed? Ann Rheum Dis 2001;60:841-5.
23. Lanting CI, Van Wouwe JP, Reijneveld SA. Infant milk feeding practices in the Netherlands and associated factors. Acta Paediatr 2005;94:935-42.

24. Verstappen SM. Rheumatoid arthritis and work: The impact of rheumatoid arthritis on absenteeism and presenteeism. Best Pract Res Clin Rheumatol 2015;29:495-511.
25. Hansen SM, Hetland ML, Pedersen J, Ostergaard M, Rubak TS, Bjorner JB. Work ability in rheumatoid arthritis patients: a register study on the prospective risk of exclusion and probability of returning to work. Rheumatology (Oxford) 2017;56:1135-43.
26. Verstappen SM, Jacobs JW, Huisman AM, van Rijthoven AW, Sokka T, Bijlsma JW. Functional Health Assessment Questionnaire (HAQ) and Psychological HAQ Are Associated with and Predicted by Different Factors in Rheumatoid Arthritis. J Rheumatol 2007;34:1837-40.
27. Gibbs BG, Forste R. Socioeconomic status, infant feeding practices and early childhood obesity. Pediatr Obes 2014;9:135-46.
28. Baerug A, Laake P, Loland BF, Tylleskar T, Tufte E, Fretheim A. Explaining socioeconomic inequalities in exclusive breast feeding in Norway. Arch Dis Child 2017;102:708-14.
29. Logan C, Zittel T, Striebel S, Reister F, Brenner H, Rothenbacher D, et al. Changing Societal and Lifestyle Factors and Breastfeeding Patterns Over Time. Pediatrics 2016;137.
30. Notley C, Blyth A, Craig J, Edwards A, Holland R. Postpartum smoking relapse--a thematic synthesis of qualitative studies. Addiction 2015;110:1712-23.
31. Simmons VN, Sutton SK, Quinn GP, Meade CD, Brandon TH. Prepartum and postpartum predictors of smoking. Nicotine Tob Res 2014;16:461-8.
32. Napierala M, Mazela J, Merritt TA, Florek E. Tobacco smoking and breastfeeding: Effect on the lactation process, breast milk composition and infant development. A critical review. Environ Res 2016;151:321-38.
33. Lefebvre F, Ducharme M. Incidence and duration of lactation and lactational performance among mothers of low-birth-weight and term infants. CMAJ 1989;140:1159-64.
34. Pineda RG. Predictors of breastfeeding and breastmilk feeding among very low birth weight infants. Breastfeed Med 2011;6:15-9.
35. Flacking R, Nyqvist KH, Ewald U, Wallin L. Long-term duration of breastfeeding in Swedish low birth weight infants. J Hum Lact 2003;19:157-65.
36. Adair LS, Popkin BM. Low birth weight reduces the likelihood of breast-feeding among Filipino infants. J Nutr 1996;126:103-12.
37. Clowse ME, Forger F, Hwang C, Thorp J, Dolhain RJ, van Tubergen A, et al. Minimal to no transfer of certolizumab pegol into breast milk: results from CRADLE, a prospective, postmarketing, multicentre, pharmacokinetic study. Ann Rheum Dis 2017;76:1890-6.



General discussion

8

This thesis provides more knowledge on the impact of maternal rheumatoid arthritis (RA) on pregnancy and outcome. Moreover, it offers useful new insights into the unresolved topics in patients with RA and their offsprings. In this general discussion, the findings of this thesis will be described as followed: new insights, clinical implications, strength and limitations of the studies described in this thesis, and the generalizability. This chapter ends with the recommendations for future research.

NEW INSIGHTS

RA disease activity

Although RA disease activity might improve during pregnancy, this improvement occurs less often and with less magnitude than previously thought [1]. At the same time, female patients with RA (and their rheumatologists) often desire to taper medication during pregnancy. In addition, current guidelines advise to discontinue e.g. some biological agents during gestation [2], while clinical guidelines on tapering antirheumatic drugs during pregnancy are absent. Since RA disease activity improves in approximately half of the patients[1], one of the aims of this thesis was to identify female RA patients who were more or less likely to improve during pregnancy.

New insights on RA disease activity:

- The improvement of RA disease activity in one pregnancy is, in contrast to previous assumptions, not predictive for improvement in following pregnancies. However, a flare postpartum is predictive for a subsequent flare.
- Low RA disease activity in the first trimester, the absence of autoantibodies, and not using prednisone in the first trimester are associated with low disease activity and remission at the end of pregnancy.

Short- and long-term consequences

It was already known that high RA disease activity might have an impact on the pregnancy outcome. Children born to women with active RA have a less fortunate start, with increased risk of lower birth weight and rapid catch-up growth in weight[3, 4], both associated with an increased risk for cardiovascular and metabolic disease in later life[5, 6]. Epigenetic processes, like DNA methylation, are thought to be one of the mechanisms underlying the associations between lower birth weight and later-life health[7, 8]. Furthermore, animal studies have shown that an excess of maternal glucocorticoids during pregnancy, is associated with altered HPA axis activity with higher plasma cortisol levels in the offspring, higher blood pressure and hyperglycemia in later life[9-12]. Since RA may negatively impact the later-life health of the

offspring[3], breastfeeding might be utmost important. Breastmilk is the most optimal infant nutrition[13, 14], and has multiple benefits for the long-term health of the child[15-21].

The long-term health outcomes of children born to women with RA, were relatively unexplored and needed to be elucidated. Therefore, another aim of this study was to determine some of the short- (e.g. lactation) and long-term consequences of maternal RA, RA disease activity, and medication use during pregnancy on the offspring.

New insights on short- and long-term consequences:

- Children born to women with RA have a different DNA methylation profile compared with children born to mothers from the general population. The differentially methylated CpGs are associated with, or located near genes that are associated with cardiovascular and metabolic disease.
- Antenatal prednisone exposure and maternal RA disease activity are not associated with chronically elevated cortisol and cortisone concentrations, nor with an altered body composition or higher blood pressure in childhood.
- Women with RA breastfeed their offspring less and with a shorter duration compared to women from the general population. Notably, a considerable amount of patients with RA discontinue breastfeeding unnecessarily by incorrectly considering the use of some medication incompatible with breastfeeding.

CLINICAL IMPLICATIONS

With the findings of this thesis, new, valuable insights have been provided regarding the impact of RA during pregnancy on mother and child. Similar to healthy women, young female RA patients also desire to conceive. Nowadays, attention for care in patients with RA who wish to conceive is gradually increasing.

RA disease activity

This thesis identified that there are predictive factors present before, and in early pregnancy, that are associated with lower disease activity at the end of pregnancy. The disease course in a previous pregnancy is, in contrast to previous assumptions[22], not a predictor for a subsequent pregnancy. Therefore, it is not reasonable to rely on disease activity in a previous pregnancy. However it is possible to anticipate a flare after delivery based on a previous flare postpartum.

This thesis did not study tapering in particular, and more research into this field is necessary. However, the combination of clinical factors before or in early pregnancy, that have been identified in this thesis, might be useful in the clinical practice for the adjustment

of antirheumatic medication during pregnancy. Women with low disease activity in the first trimester are likely to maintain low disease activity or achieve remission in the third trimester, independent of their autoantibody status and prednisone use. In these patients careful tapering of antirheumatic drugs may be feasible.

Short- and long-term consequences

This thesis has shown that offsprings born to women with RA during pregnancy have a different DNA methylation profile compared with children born to healthy women. Altered methylation is associated in other studies with cardiovascular and metabolic disease in adulthood [7,8,10,23]. In our study, some of the differentially methylated CpGs have directly been associated with, or are located near genes that have been associated with cardiovascular and metabolic disease. At the age of approximately 7, the DNA methylation profile was not associated with an altered body composition in our study. However, it is still possible that there will be consequences for these children in adulthood.

Although RA might impact the pregnancy outcome not only on short-term, but also on long-term, the clinical consequences for the offsprings on the long-term have not been demonstrated (yet). Therefore, patients may be reassured that the long-term consequences of maternal RA during pregnancy for their offspring are probably limited.

In addition, this study has revealed that low- dose antenatal prednisone exposure and maternal RA disease activity during pregnancy are not associated with chronically elevated cortisol and cortisone concentrations, an altered body composition, and/or a higher blood pressure in the offspring. Maternal prednisone use during pregnancy is associated in other studies with short-term effects on the pregnancy outcome, as reviewed in chapter 2 of this thesis. In our study we have shown that the long-term impact of low-dose antenatal prednisone exposure is limited. Especially in patients for whom TNF inhibitors are not available or not desired, treatment strategies including low-dose prednisone should be preferred. In addition, patients using low-dose prednisone should be reassured concerning the long-term consequences for their offspring.

Regarding lactation, the results of this study revealed a largely neglected area that requires additional attention, where there is a considerable amount of profit that can be gained. In RA fewer women breastfeed their offspring compared with women from the general population. A significant number of patients reported discontinuation of lactation due to the restart of medication, while they restarted medication that was considered compatible with lactation at the time our study was conducted[24]. Breastmilk might particularly be beneficial for offsprings born to women with RA since breastfeeding in infancy is associated with e.g. a lower occurrence of cardiovascular risk factors in adulthood[25]. Education of RA patients on the safety of using various antirheumatic medication during lactation may be valuable and may encourage patients breastfeeding their infant for a longer period of time.

STRENGTH AND LIMITATIONS

This thesis is based on the PARA-, FEPRA-, and HAIR-study, all nationwide prospective cohort studies from the Netherlands (described in more detail in the general introduction). In this part of the general discussion, the strength and limitations of these three studies will be discussed.

General limitations

As for all observational studies, certain limitations regarding internal and external validity might also apply to the studies described in this thesis. Internal validity refers to the strength of inferences from a study, and external validity refers to the generalizability of the results[26]. In prospective cohort studies, problems in internal validation include random errors, systematic errors (e.g. selection bias, information bias), and confounding. Random errors were strived to be limited by collecting relatively large sample sizes. Selection bias might have multiple causes, like a healthy worker effect or loss to follow-up. Testing the baseline characteristics between participants and non-participants resulted in no statistical differences. Information bias, for example due to interviewer or recall bias, was strived to be limited by using standardized protocols for data collection, standardized questionnaires, and trained researchers. Confounding may occur when baseline characteristics or prognostic factors differ between the compared groups. Even though it is difficult to eliminate all confounders, using a comparable control group, and correcting for possible confounders in the statistical models improve the internal validation of a study[27]. Finally, in prospective cohort studies exposures precede the outcome, proving a temporal sequence. However, this does not automatically prove a causal relationship. Therefore, careful interpretation of the results is important.

PARA-study

The PARA-study was the first large nationwide prospective cohort study on the impact of pregnancy on RA, and the impact of RA on the pregnancy outcome.

The main strength of the PARA-study is that all pregnancies in this large cohort were prospectively followed from conception (if possible) until 26 weeks postpartum. Patients were visited at their home address, increasing the compliance, resulting that in majority of the patients all visits were completed. The visits were performed by the same research assistants, limiting variations in measurements. Another strength is that in the PARA-study the RA disease activity was calculated using the DAS28-CRP with 3 variables, which is considered to be the most reliable scoring method for RA disease activity during pregnancy [28].

The main limitation of the PARA-study is that the study was conducted a decade ago, when TNF inhibitors were not prescribed during pregnancy. Participants of the PARA-study only used prednisone, sulfasalazine, and hydroxychloroquine during pregnancy. Another limitation was that, in the PARA-study, patients were enrolled between 2002 and 2008, when American College of Rheumatology (ACR) 1987 revised criteria for RA[29] applied. However, in the past decade, the ACR/ European League Against Rheumatism (EULAR) 2010 classification criteria for RA were introduced [30], resulting in earlier diagnosis. The 2010 criteria have a higher sensitivity and a lower specificity compared with the 1987 criteria[31, 32], and approximately 90% of the patients of the patients that fulfill the 1987 criteria, also fulfill the 2010 criteria[32]. Therefore, the vast majority of the PARA-participants will probably fulfill the 2010 criteria.

FEPRA-study

For the FEPRA-study, a few years later, patients were asked to visit the Sophia Children's Hospital in Rotterdam once with their children. This study focused on the long-term consequences of maternal RA during pregnancy on the offspring. During this visit, in all children, anthropometrics were measured and a Dual-Energy X-ray Absorptiometry (DXA)-scan was performed. In addition, saliva and fasting blood samples were collected.

The main strength of the FEPRA-study was that the study population was a relatively large and unique cohort of children born to women with RA, who were followed in a large prospective study before, during and after their pregnancy.

The main limitation of the FEPRA-study was that only 55% of the PARA-study participants agreed to participate with the FEPRA-study. The majority of the patients declined participation due to the distance to the hospital. Nevertheless, there was no statistical difference in independent variables between the participating and non-participating group, decreasing the likelihood of selection bias.

HAIR-study

A few years later, PARA- study participants were approached once more to participate in another follow-up study with their children, the HAIR-study. In this study, children between the age of 5 and 16 were included to study the effects of antenatal prednisone exposure. Participants throughout the Netherlands were visited at their home address where a tuft of hair from the children and anthropometric data from children were collected.

The main strength of the HAIR-study was that this was again a relatively large follow-up study of a previous large prospective cohort study. In addition, in this study, scalp hair was used to measure long-term steroid levels, which is considered to be the most reliable method.

The main limitation of the HAIR-study was that only 117 (46.4%) from the invited patients agreed to participate with their child. The main reason for refusing participation was that the parents were "tired" of participating in studies, and that they felt that the investigations

were too much of a burden for their child. However, there were nog significant differences in baseline characteristics between participants and non-participants. Therefore it is unlikely that selection bias influenced the results of this study.

GENERALIZABILITY

After summarizing the most important findings of this thesis and discussing the strength and limitations of our studies in the preceding sections, it is necessary to look into the generalizability of our results. Medical research is mostly performed to increase knowledge about a disease and to improve patient care by using the results. Research findings should be implemented into the daily practice to improve patient care. In order to achieve implementation, the generalizability of the results is essential. As for our study, not only the generalizability of the results to RA patients from the general population, but also generalizability of the results to patients with other autoimmune diseases is utmost important.

Since our studies were nationwide, and patients were included from both urban and rural areas, and from every socioeconomic status, we think that the majority of the results are generalizable to female patients with RA in the Netherlands.

However, there are some differences between our study and the current practice. First, our study population consisted of female RA patients who fulfilled the ACR 1987 revised criteria, while currently the ACR/EULAR 2010 criteria are applied. Nevertheless, as mentioned before, this probably did not influence the results of this thesis.

Second, at the time our study was conducted, TNF inhibitors were not prescribed to RA patients during pregnancy, and TNF inhibitors were not considered compatible with lactation. Therefore, we cannot draw any conclusions on the short-and long-term consequences of TNF inhibitors on the offspring.

This study does not only provide useful insights for female patients with RA, but also for female patients with other rheumatic diseases who wish to conceive. Juvenile idiopathic arthritis (JIA) is a pediatric rheumatic disease with an onset in childhood[33]. In majority of these patients, the disease remains active until adulthood[34]. Although JIA is a more heterogeneous autoimmune disease than RA, the pathological basis of these two disease share similarities (especially with polyarticular JIA)[35]. Psoriatic arthritis (PsA) has a peak prevalence between the age of 40 and 50 years, however, the disease may also occur in younger patients[36]. Even though there are substantial differences in the pathogenesis of PsA and RA[37], in both diseases the pathophysiology involves a chronic inflammation mediated by pro-inflammatory cytokines[38]. As both JIA and PsA patients share common pathophysiological mechanisms and have comparable treatment options during pregnancy, it is plausible that

the results of our study on short-and long-term consequences for the offspring are also useful for these patients.

In addition, the generalizability of our study is not limited for patients with rheumatic diseases, but extends to female patients with other autoimmune diseases who wish to conceive, and for patients who use corticosteroids during pregnancy. Female patients with an inflammatory bowel disease (IBD) are an excellent example of patients that can also benefit from this research. IBD has a high prevalence in women of childbearing age[39], and shares common pathogenic mechanisms with rheumatic diseases. IBD treatment during pregnancy is also comparable with the treatment of RA during pregnancy, including the use of corticosteroids. Therefore, the results of our studies may also be valuable for IBD patients.

RECOMMENDATIONS FOR FUTURE RESEARCH BASED ON THIS THESIS

This thesis has revealed several important associations between maternal RA during pregnancy and short- and long-term consequences for mother and child. However, there still is a lack of prospective studies on this subject. The results from this thesis highlight the importance of conducting more studies to evaluate the impact of RA during pregnancy on mother and child. Several recommendations for future research, that are derived from our results, are discussed below.

In the current era, treat-to-target approaches are applied for the treatment of RA (not necessarily during pregnancy) [40]. Furthermore, treatment guidelines for patients with RA during pregnancy include the use of TNF inhibitors [2]. Prospective follow-up studies of pregnant patients using TNF inhibitors during pregnancy and their offsprings are upcoming. However, studies on long-term consequences for the offspring are lacking. First of all, future studies could reveal whether more intensive antirheumatic treatment before and during pregnancy may lead to improved pregnancy outcomes. Another recommendation for future research include studying the long-term consequences of antenatal exposure to TNF inhibitors, such as differential DNA methylation.

In 2016, a EULAR task force has reported points to consider for use of antirheumatic drugs before pregnancy, during pregnancy, and during lactation[2]. However, clinical guidelines on tapering medication during pregnancy in patients with RA are lacking. This thesis provides clinical characteristics at the beginning of pregnancy that are associated with low disease activity or remission in the third trimester. It will be interesting to perform a prospective study on tapering medication in pregnant patients with RA, using the characteristics that have been defined in this thesis. With the results of that prospective study, guidelines on

tapering antirheumatic drugs during pregnancy may be developed. Subsequently, it would be interesting to observe the results of these guidelines.

Educating physicians in the most optimal way into the subject of pregnancy and lactation in patients with RA will be challenging. More research is necessary in order to develop guidelines for the management of RA during pregnancy that are applicable in different countries around the world. To guarantee successful implementation of these guidelines, it is also recommended to study the most optimal implementation technique. Subsequently, it would be interesting to observe the results of these guidelines.

Although our study on DNA methylation in children born to women with RA was in its kind a relatively large study, an epigenetic study in 80 children (and 354 controls) is still small. Nevertheless, we did find 147 CpGs that were significantly different between children born to women with RA and children born to women from the general population. A recommendation for future research is to replicate our findings in another cohort of children born to women with RA. In addition, even though we had enough power to find differences between the two groups, our study did not have enough power to relate the differences to RA disease activity and/or medication use. An international collaboration will result in a study with a larger population of children born to women with RA (or other autoimmune disease).

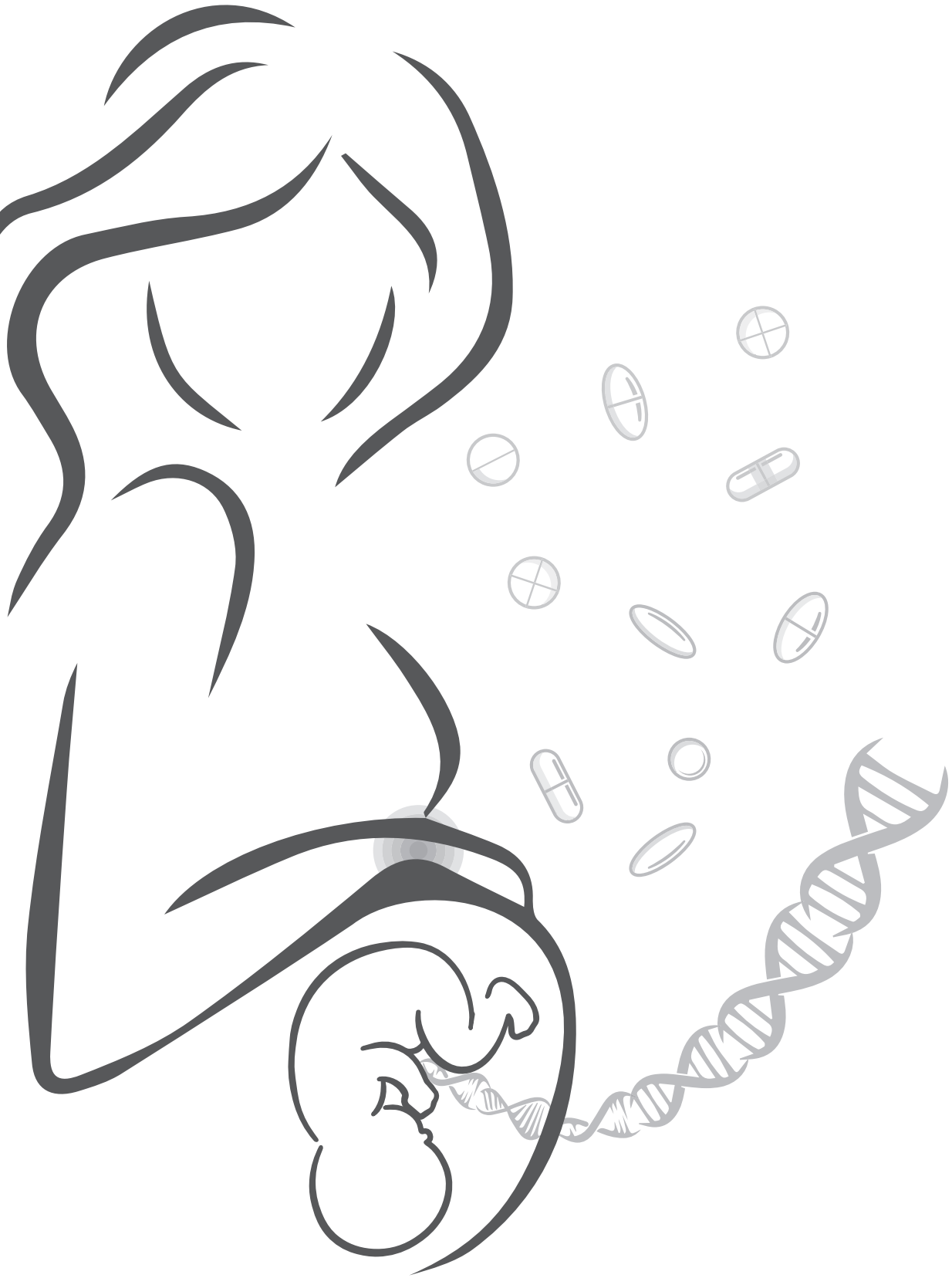
This thesis has revealed that maternal prednisone use and maternal RA disease activity is not associated with impaired HPA axis activity or an altered body composition in approximately 10-years-old offsprings. However, it might very well be possible that at this age the children are too young to draw any definite conclusions on their risk of cardiovascular disease in adulthood. This thesis has shown that children born to women with RA have significant differences in the methylation of CpGs that are located near genes associated with cardiovascular and metabolic disease. Therefore, another suggestion for further research is to continue the follow-up of these children until early adulthood to assess their risk of cardiovascular and metabolic disease, preferably in relation to the disease activity during pregnancy and medication use.

Finally, although somewhat outside of the scope of this thesis, studies on male patients with RAin the fertile age are lacking. Studies on RA disease activity and medication use in male RA patients who try to conceive may elucidate the impact of paternal RA on pregnancy and the pregnancy outcome.

REFERENCES

1. de Man YA, Dolhain RJ, van de Geijn FE, Willemsen SP, Hazes JM. Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study. Arthritis Rheum 2008;59:1241-8.
2. Gotestam Skorpen C, Hoeltzenbein M, Tincani A, Fischer-Betz R, Elefant E, Chambers C, et al. The EULAR points to consider for use of antirheumatic drugs before pregnancy, and during pregnancy and lactation. Ann Rheum Dis 2016;75:795-810.
3. de Man YA, Hazes JM, van der Heide H, Willemsen SP, de Groot CJ, Steegers EA, et al. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: results of a national prospective study. Arthritis Rheum 2009;60:3196-206.
4. de Steenwinkel FD, Hokken-Koelega AC, de Ridder MA, Hazes JM, Dolhain RJ. Rheumatoid arthritis during pregnancy and postnatal catch-up growth in the offspring. Arthritis Rheumatol 2014;66:1705-11.
5. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. JAMA 2009;301:2234-42.
6. Ong KK, Dunger DB. Perinatal growth failure: the road to obesity, insulin resistance and cardiovascular disease in adults. Best Pract Res Clin Endocrinol Metab 2002;16:191-207.
7. Turan N, Ghalwash MF, Katari S, Coutifaris C, Obradovic Z, Sapienza C. DNA methylation differences at growth related genes correlate with birth weight: a molecular signature linked to developmental origins of adult disease? BMC Med Genomics 2012;5:10.
8. Hogg K, Price EM, Hanna CW, Robinson WP. Prenatal and perinatal environmental influences on the human fetal and placental epigenome. Clin Pharmacol Ther 2012;92:716-26.
9. Levitt NS, Lindsay RS, Holmes MC, Seckl JR. Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. Neuroendocrinology 1996;64:412-8.
10. Cottrell EC, Seckl JR. Prenatal stress, glucocorticoids and the programming of adult disease. Front Behav Neurosci 2009;3:19.
11. Singh RR, Cuffe JS, Moritz KM. Short- and long-term effects of exposure to natural and synthetic glucocorticoids during development. Clin Exp Pharmacol Physiol 2012;39:979-89.
12. Seckl JR. Prenatal glucocorticoids and long-term programming. Eur J Endocrinol 2004;151 Suppl 3:U49-62.
13. Liu J, Leung P, Yang A. Breastfeeding and active bonding protects against children's internalizing behavior problems. Nutrients 2013;6:76-89.
14. Anderson GC, Moore E, Hepworth J, Bergman N. Early skin-to-skin contact for mothers and their healthy newborn infants. Birth 2003;30:206-7.
15. Daly A, Pollard CM, Phillips M, Binns CW. Benefits, barriers and enablers of breastfeeding: factor analysis of population perceptions in Western Australia. PLoS One 2014;9:e88204.
16. Gharib S, Fletcher M, Tucker R, Vohr B, Lechner BE. Effect of Dedicated Lactation Support Services on Breastfeeding Outcomes in Extremely-Low-Birth-Weight Neonates. J Hum Lact 2017;in press:890334417741304.
17. Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, et al. Breastfeeding and maternal and infant health outcomes in developed countries. Evid Rep Technol Assess (Full Rep) 2007;1:1-186.
18. Oddy WH. Breast feeding and childhood asthma. Thorax 2009;64:558-9.
19. Oddy WH. The long-term effects of breastfeeding on asthma and atopic disease. Adv Exp Med Biol 2009;639:237-51.
20. Owen CG, Martin RM, Whincup PH, Smith GD, Cook DG. Effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. Pediatrics 2005;115:1367-77.
21. Owen CG, Whincup PH, Kaye SJ, Martin RM, Davey Smith G, Cook DG, et al. Does initial breastfeeding lead to lower blood cholesterol in adult life? A quantitative review of the evidence. Am J Clin Nutr 2008;88:305-14.
22. Barrett JH, Brennan P, Fiddler M, Silman AJ. Does rheumatoid arthritis remit during pregnancy and relapse postpartum? Results from a nationwide study in the United Kingdom performed prospectively from late pregnancy. Arthritis Rheum 1999;42:1219-27.
23. Reynolds RM. Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis--2012 Curt Richter Award Winner. Psychoneuroendocrinology 2013;38:1-11.

24. Ostensen M, Khamashta M, Lockshin M, Parke A, Brucato A, Carp H, et al. Anti-inflammatory and immunosuppressive drugs and reproduction. Arthritis Res Ther 2006;8:209.
25. Parikh NI, Hwang SJ, Ingelsson E, Benjamin EJ, Fox CS, Vasan RS, et al. Breastfeeding in infancy and adult cardiovascular disease risk factors. Am J Med 2009;122:656-63 e1.
26. Carlson MDA, Morrison RS. Study design, precision, and validity in observational studies. Journal of palliative medicine 2009;12:77-82.
27. Lu CY. Observational studies: a review of study designs, challenges and strategies to reduce confounding. Int J Clin Pract 2009;63:691-7.
28. de Man YA, Hazes JM, van de Geijn FE, Krommenhoek C, Dolhain RJ. Measuring disease activity and functionality during pregnancy in patients with rheumatoid arthritis. Arthritis Rheum 2007;57:716-22.
29. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
30. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62:2569-81.
31. Berglin E, Dahlqvist SR. Comparison of the 1987 ACR and 2010 ACR/EULAR classification criteria for rheumatoid arthritis in clinical practice: a prospective cohort study. Scand J Rheumatol 2013;42:362-8.
32. van der Linden MP, Knevel R, Huizinga TW, van der Helm-van Mil AH. Classification of rheumatoid arthritis: comparison of the 1987 American College of Rheumatology criteria and the 2010 American College of Rheumatology/European League Against Rheumatism criteria. Arthritis Rheum 2011;63:37-42.
33. Fernandes S Fau - Fernandes S, Gomes Jam Fau - Gomes JAM, Gomes Sm Fau - Gomes SM, Martins F Fau - Martins F. Juvenile idiopathic arthritis: the transition to adulthood.in press:P193.
34. Oliveira-Ramos F, Eusébio M, F MM, Mourão AF, Furtado C, Campanilho-Marques R, et al. Juvenile idiopathic arthritis in adulthood: fulfilment of classification criteria for adult rheumatic diseases, long-term outcomes and predictors of inactive disease, functional status and damage. RMD Open 2016;2.
35. Prahalad S Fau - Prahalad S, Glass Dn Fau - Glass DN. Is juvenile rheumatoid arthritis/juvenile idiopathic arthritis different from rheumatoid arthritis? ;in press:303-10.
36. Liu JT, Yeh HM, Liu SY, Chen KT. Psoriatic arthritis: Epidemiology, diagnosis, and treatment. World J Orthop 2014;5:537-43.
37. FitzGerald O, Winchester R. Psoriatic arthritis: from pathogenesis to therapy. Arthritis Res Ther 2009;11:214.
38. Coates LC, FitzGerald O, Helliwell PS, Paul C. Psoriasis, psoriatic arthritis, and rheumatoid arthritis: Is all inflammation the same? Semin Arthritis Rheum 2016;46:291-304.
39. Hoekstra J, van Roon AHC, Bekkering FC, van Tilburg AJP, West RL. Decision making and outcome of pregnancies in female patients with inflammatory bowel disease: findings from a community-based practice. Eur J Gastroenterol Hepatol 2018;in press.
40. Smolen JS, Landewe R, Breedveld FC, Dougados M, Emery P, Gaujoux-Viala C, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. Ann Rheum Dis 2010;69:964-75.



Summary

9

Rheumatoid arthritis (RA) is a common disease, also affecting women in the childbearing age. It was already known that high RA disease activity and prednisone use influence the pregnancy outcome. However, it was not known which women were prone to have high disease activity during pregnancy and what kind of impact this might have on the offsprings. Therefore, the aims of this thesis were to identify clinical factors associated with improvement of the disease activity during pregnancy, and to identify the possible short- and long-term consequences of RA, RA disease activity and prednisone use on the offsprings.

Chapter 1

This chapter introduces the topics explored in this thesis. It provides an overview of RA in general, during pregnancy, and during lactation. Furthermore, it describes the developmental origins of health and disease (DOHaD) hypothesis, explaining the lifelong consequences of adverse circumstances during pregnancy on the offspring. In addition, it gives an overview of the role of cortisol and the importance of epigenetics within this subject. Finally the designs of the PARA, FEPRA, and HAIR-study are described.

Chapter 2

In this chapter, multiple aspects on RA and pregnancy are reviewed. It describes among others, fertility in patients with RA, the RA disease course during and after pregnancy, pregnancy outcomes, effects on the offspring. In addition, it reviews the pathogenic mechanism underlying the improvement of RA during pregnancy, and the treatment of RA during pregnancy. Finally, it provides practical approaches to patients with RA who wish to conceive.

Chapter 3

This chapter illustrates the RA disease activity during and after two subsequent pregnancies. The course of the RA disease activity (improvement, deterioration, or a combination) during a first pregnancy was not associated with the disease course in a second pregnancy. However, a flare postpartum after a first pregnancy was associated with a subsequent flare. Therefore, in a subsequent pregnancy, treatment should not be based on the disease course in a previous pregnancy. However, a deterioration of the disease activity postpartum may be anticipated based on a previous flare.

Chapter 4

The objective of this chapter was to determine a combination of clinical factors at the beginning of pregnancy, associated with low disease activity and remission of RA in the third trimester during pregnancy. RA patients who had a low disease activity in the first

trimester (irrespective of autoantibody status or prednisone use) were likely to have low disease activity or remission in the third trimester. Women with higher disease activity in the first trimester who were taking prednisone and/or expressed autoantibodies were less likely to have low disease activity and remission in the last trimester. In addition, RA patients with higher disease activity who were not taking prednisone and did not express autoantibodies, still had a fair chance for low disease activity in the third trimester. These findings may be useful for daily clinical practice, or for future studies.

Chapter 5

Epigenetics are thought to be one of the mechanisms underlying the earlier mentioned DOHaD hypothesis. DNA methylation is an epigenetic modification, which can be defined as tissue specific changes in DNA without changing the underlying DNA sequence. Changes in DNA methylation may result in altered gene expression. DNA methylation occurs at cytosine (C) bases where they are located near guanine (G) bases with a phosphate (p) group in the middle. These sites where methylation is measured are called CpGs.

This chapter investigates the influence of maternal RA during pregnancy on the DNA methylation profile of their offspring. Children born to women with RA had a different DNA methylation profile compared with children born to mothers from the general population. In total 147 CpGs were significantly different between these groups. From the top 5 CpGs, 2 were associated in another study with myocardial infarction. Other significant CpGs were located near genes that are associated with e.g. cardiovascular and metabolic disease. These findings support the hypothesis that RA during pregnancy might have life-long consequences for the offspring.

Chapter 6

In this chapter the long-term consequences for children of maternal prednisone use and RA disease activity during pregnancy were studied. Antenatal prednisone exposure and RA disease activity were not associated with chronically elevated cortisol and cortisone levels in children born to women with RA. In addition, antenatal prednisone exposure and RA disease activity were not associated with an altered body composition or a higher blood pressure in childhood. These findings are reassuring for women who use prednisone during pregnancy and their doctors.

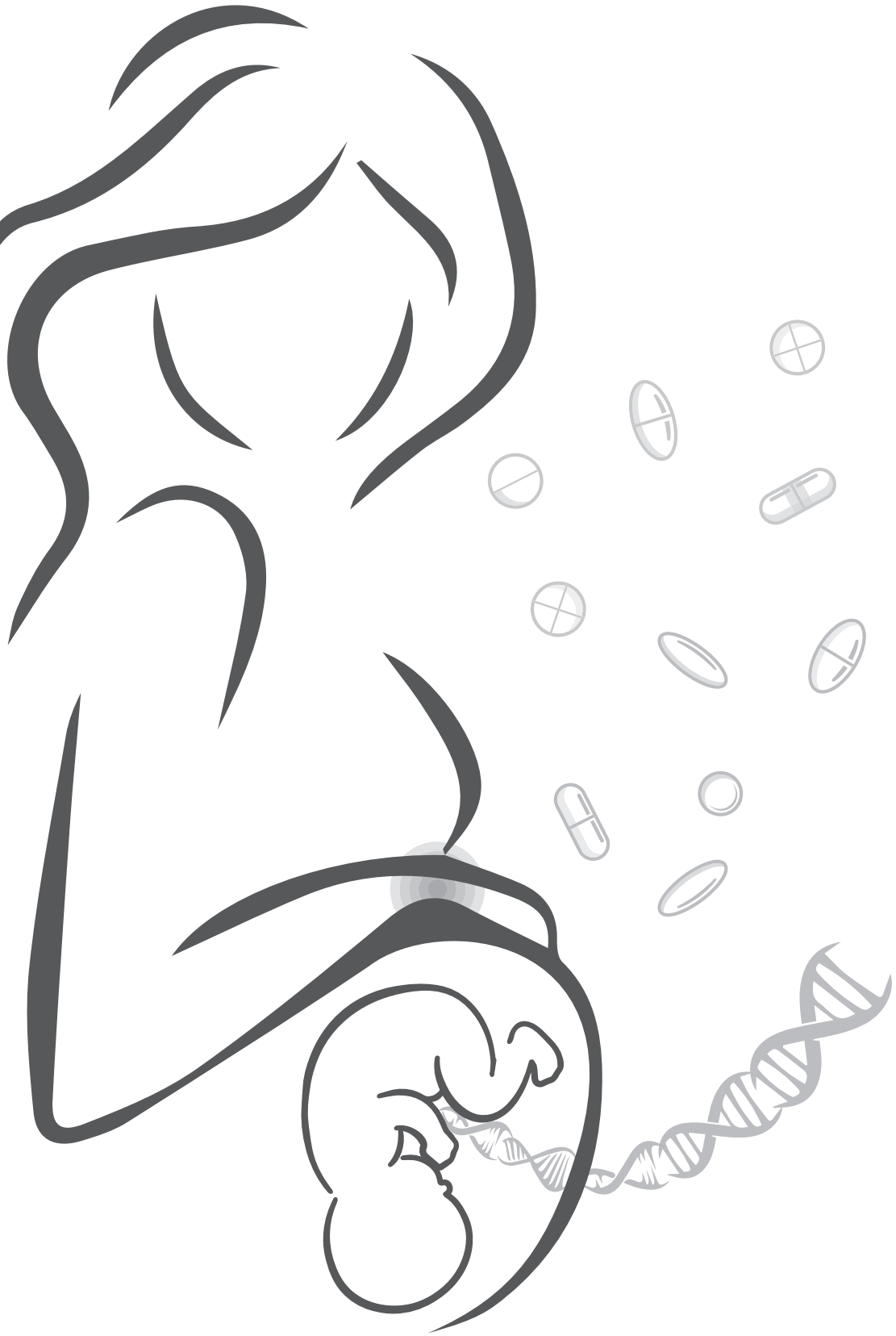
Chapter 7

This chapter focusses on lactation in female patients with RA. Women with RA breastfeed their offspring less often and with a shorter duration compared with women from the general population. The main reason of discontinuation was the restart of medication, however half of these patients discontinued unnecessarily since they restarted medication

that was considered compatible with lactation. In addition, a worse functional status expressed by a higher HAQ, the presence of autoantibodies, and smoking were associated with discontinuation of breastfeeding within 12 weeks postpartum. Education of patients, doctors, and nurses on the use of medication during lactation might enable more patients to breastfeed their offspring for a longer period of time.

Chapter 8

Finally, in this chapter, the general discussion, the new insights based on the findings in this thesis, and the clinical implications are described. Furthermore, the strength and limitations of the PARA, FEPRA, and HAIR-study, and the generalizability of the results are discussed. This chapter ends with the recommendations for future research, based on the findings in this thesis.



Samenvatting

10

Reumatoïde artritis (RA) is een veelvoorkomende ziekte, waar ook vrouwen in de vruchtbare leeftijd al aan kunnen lijden. Uit eerder onderzoek is gebleken dat een hoge ziekteactiviteit van RA en het gebruik van prednison door de moeder tijdens de zwangerschap invloed hebben op de zwangerschapsuitkomsten. Echter, is het nog niet onderzocht welke vrouwen meer kans hebben op lage of hoge ziekteactiviteit gedurende de zwangerschap en wat de impact hiervan kan zijn op hun kinderen. Daarom heeft dit proefschrift als doelen: het identificeren van klinische factoren die geassocieerd zijn met de activiteit van RA tijdens de zwangerschap en het identificeren van de mogelijke korte en lange termijn gevolgen van het hebben van RA, de ziekteactiviteit van RA en het effect van het gebruik van prednison tijdens de zwangerschap op de kinderen.

Hoofdstuk 1

Dit hoofdstuk introduceert de onderwerpen die onderzocht zijn in dit proefschrift. Het geeft een overzicht van RA in het algemeen, RA gedurende de zwangerschap en RA tijdens het geven van borstvoeding. Daarnaast worden in dit hoofdstuk de behandelopties van RA tijdens de zwangerschap en tijdens het geven van borstvoeding besproken. Vervolgens wordt in hoofdstuk 1 de "developmental origins of health and disease" (DOHaD) hypothese beschreven, welke een verklaring geeft voor de levenslange gevolgen op de kinderen van bepaalde omstandigheden gedurende de zwangerschap . Volgens deze hypothese, kan blootstelling aan meerdere ongunstige omstandigheden in de baarmoeder leiden tot hart- en vaatziekten en metabole ziektes (zoals bijvoorbeeld diabetes mellitus type 2) in het latere leven. Hoofdstuk 1 licht ook de rol van het stress-hormoon cortisol en het belang van epigenetica als mogelijke verklaring voor de DOHaD hypothese toe. Tenslotte wordt de opzet van de PARA, FEPRA en HAIR studie beschreven.

Hoofdstuk 2

In dit hoofdstuk worden meerdere aspecten van RA en zwangerschap besproken. Het beschrijft onder andere de vruchtbaarheid van patiënten met RA, vrouwen met RA doen er langer over om zwanger te worden dan gezonde vrouwen. Daarnaast wordt het beloop van de RA ziekteactiviteit gedurende en na de zwangerschap besproken. Tijdens de zwangerschap verbetert de ziekteactiviteit, maar in minder vrouwen en in mindere mate dan op basis van eerder onderzoek werd gedacht. Na de bevalling verslechtert de ziekteactiviteit in de meerderheid van de RA patiënten. In hoofdstuk 2 worden ook de zwangerschapsuitkomsten van vrouwen met RA beschreven. Volgens sommige studies hebben vrouwen met RA een iets verhoogde kans op zwangerschapsvergiftiging tijdens de zwangerschap. Ook is een hoge ziekteactiviteit van de RA geassocieerd met een grotere kans op een keizersnede en is het gebruik van prednison geassocieerd met vroeggeboorte van het kind. Verder hebben kinderen van moeders met RA een lager geboortegewicht,

ook wanneer dit gewicht gecorrigeerd wordt voor de duur van de zwangerschap.
Hoofdstuk 2 geeft ook een overzicht van de pathogenetische mechanismen die ten grondslag liggen aan de verbetering van RA gedurende de zwangerschap, zoals veranderingen in de hormonen, de aanwezigheid van autoantilichamen en de samenstelling van afweercellen.
Vervolgens beschrijft het de behandelopties van RA tijdens de zwangerschap. Tot slot geeft het praktische tips voor de behandeling en begeleiding van vrouwen met RA die een zwangerschapswens hebben.

Hoofdstuk 3

Dit hoofdstuk beschrijft het beloop van de ziekteactiviteit van vrouwen met RA die tijdens twee opeenvolgende zwangerschappen zijn vervolgd. Tijdens de zwangerschap kan de ziekteactiviteit van RA spontaan verbeteren. In oudere studies is aangetoond dat verbetering van de ziekteactiviteit in een eerste zwangerschap voorspellend is voor de verbetering in een volgende zwangerschap. Echter, is de informatie over de ziekteactiviteit in deze studies retrospectief verzameld en zijn subjectievere maten gebruikt om de ziekteactiviteit te meten. Hoofdstuk 3 geeft de resultaten weer van onderzoek naar 27 vrouwen die tijdens en na 2 zwangerschappen prospectief gevolgd zijn en waarbij de ziekteactiviteit objectief is gemeten. De resultaten laten zien dat het beloop van de ziekteactiviteit van RA (dat wil zeggen een verbetering, verslechtering of een combinatie hiervan) gedurende een eerste zwangerschap niet voorspellend is voor het ziektebeloop in een tweede zwangerschap. Echter, een opvlamming van de ziekteactiviteit na een eerste zwangerschap is wel gerelateerd aan een opvlamming na een volgende zwangerschap. In een volgende zwangerschap moet de behandeling daarom niet gebaseerd worden op het ziektebeloop in een eerdere zwangerschap. Echter, er kan wel geanticipeerd worden op een verslechtering van de ziekteactiviteit na de bevalling op basis van een eerder opvlamming.

Hoofdstuk 4

Het doel van dit hoofdstuk is om te bepalen of een combinatie van klinische factoren in het begin van de zwangerschap, geassocieerd is met een lage ziekteactiviteit of remissie van RA in het derde trimester van de zwangerschap. Zoals eerder beschreven, verbetert de ziekteactiviteit van RA in een deel van de patiënten spontaan tijdens de zwangerschap. Het is echter nog niet bekend in welke vrouwen de kans het grootst is dat de ziekteactiviteit verbetert. In hoofdstuk 4 beschrijft vrouwen die tijdens en na 190 zwangerschappen zijn gevolgd. De volgende klinische factoren zijn onderzocht in de statistische analyses: de ziekteactiviteit van RA in het 1ste trimester, het gebruik van prednison in het 1ste trimester, het gebruik van sulfasalazine in het 1ste trimester, pariteit, het gebruik van methotrexaat in het verleden, de aanwezigheid van autoantilichamen, de aanwezigheid van erosies en de duur van de RA.

De resultaten laten zien dat RA patiënten met een lage ziekteactiviteit in het eerste trimester meer kans hebben op lage ziekteactiviteit of remissie in het derde trimester. Dit effect is onafhankelijk van de aanwezigheid van autoantilichamen en het gebruik van prednison. Bovendien, vrouwen met een hogere ziekteactiviteit in het eerste trimester die prednison gebruiken en/of autoantilichamen in het bloed hebben, hebben minder kans op een lage ziekteactiviteit of remissie in het derde trimester. Daarentegen, vrouwen met een hogere ziekteactiviteit die geen prednison gebruiken en geen autoantilichamen in het bloed hebben, hebben toch een redelijke kans op een lage ziekteactiviteit of remissie in het derde trimester. Deze bevindingen kunnen nuttig zijn in de dagelijkse praktijk en in toekomstige onderzoeken.

Hoofdstuk 5

Dit hoofdstuk onderzoekt de invloed van RA van de moeder op de epigenetische veranderingen in het kind. Epigenetica wordt beschouwd als één van de onderliggende mechanismen van de eerder genoemde DOHaD hypothese. DNAmethylatie is een epigenetische verandering, welke beschreven kan worden als weefsel specifieke aanpassingen in het DNA zonder daarbij aanpassingen te doen in de onderliggende DNA code. Hierdoor kunnen modificaties in de DNAmethylatie leiden tot een veranderde genexpressie. DNAmethylatie vindt plaats in cytosine (C) basen, daar waar ze gelokaliseerd zijn naast guanine (G) basen met een fosfaat (p) groep tussenin. Deze locaties worden CpGs genoemd en op deze locaties kan de hoeveelheid methylatie gemeten worden.

In dit hoofdstuk zijn 80 kinderen, op een leeftijd van ongeveer 6-7 jaar, van moeders met RA vergeleken met 345 kinderen van moeders uit de algemene populatie. Dit onderzoek werd prospectief uitgevoerd. De resultaten van dit onderzoek laten zien dat kinderen van moeders met RA een ander methylatie profiel hebben dan kinderen van moeders uit de algemene populatie. In totaal zijn 147 CpGs significant anders tussen de 2 vergeleken groepen. Van de 5 meest significante CpGs zijn er 2 die in een andere studie geassocieerd zijn met het optreden van een hartinfarct. Andere significante CpGs zijn gelokaliseerd in of nabij genen die geassocieerd zijn met hart- en vaatziekten en metabole ziektes (zoals bijvoorbeeld diabetes mellitus type 2). Deze bevindingen ondersteunen de hypothese dat RA tijdens de zwangerschap levenslange gevolgen zou kunnen hebben op de gezondheid van het kind.

Hoofdstuk 6

In dit hoofdstuk worden de lange termijn gevolgen van het gebruik van prednison door de moeder en de ziekteactiviteit van RA tijdens de zwangerschap voor hun kinderen bestudeerd. Het gebruik van prednison werd geobjectiveerd door gebruik te maken van cortisol en cortison concentraties (cortison is de inactieve vorm van cortisol). Cortisol heeft

een belangrijke rol in de DOHaD hypothese. Voor dit onderzoek zijn in totaal 56 kinderen met blootstelling aan prednison en 61 kinderen zonder blootstelling aan prednison tijdens de zwangerschap geïncludeerd. Cortisol en cortison zijn gemeten in het haar, dit is een betrouwbare methode voor het meten van lange termijn concentraties. De resultaten laten zien dat blootstelling aan prednison in de baarmoeder (dit wordt antenatale blootstelling genoemd) en de ziekteactiviteit van RA van de moeder tijdens de zwangerschap niet geassocieerd zijn met chronisch verhoogde waardes van cortisol en cortison concentraties in het haar van kinderen van moeders met RA. De cortisolwaardes in het haar van kinderen van moeders met RA zijn vergelijkbaar met haar cortisolwaardes in kinderen uit de algemene populatie.

Bovendien zijn antenatale blootstelling aan prednison en ziekteactiviteit van RA niet geassocieerd met een andere lichaamssamenstelling (bijvoorbeeld een hoger BMI of een hoger vetpercentage) of een verhoogde bloeddruk op kinderleeftijd. Deze bevindingen zijn geruststellend voor vrouwen die prednison gebruiken tijdens de zwangerschap en hun behandelende artsen.

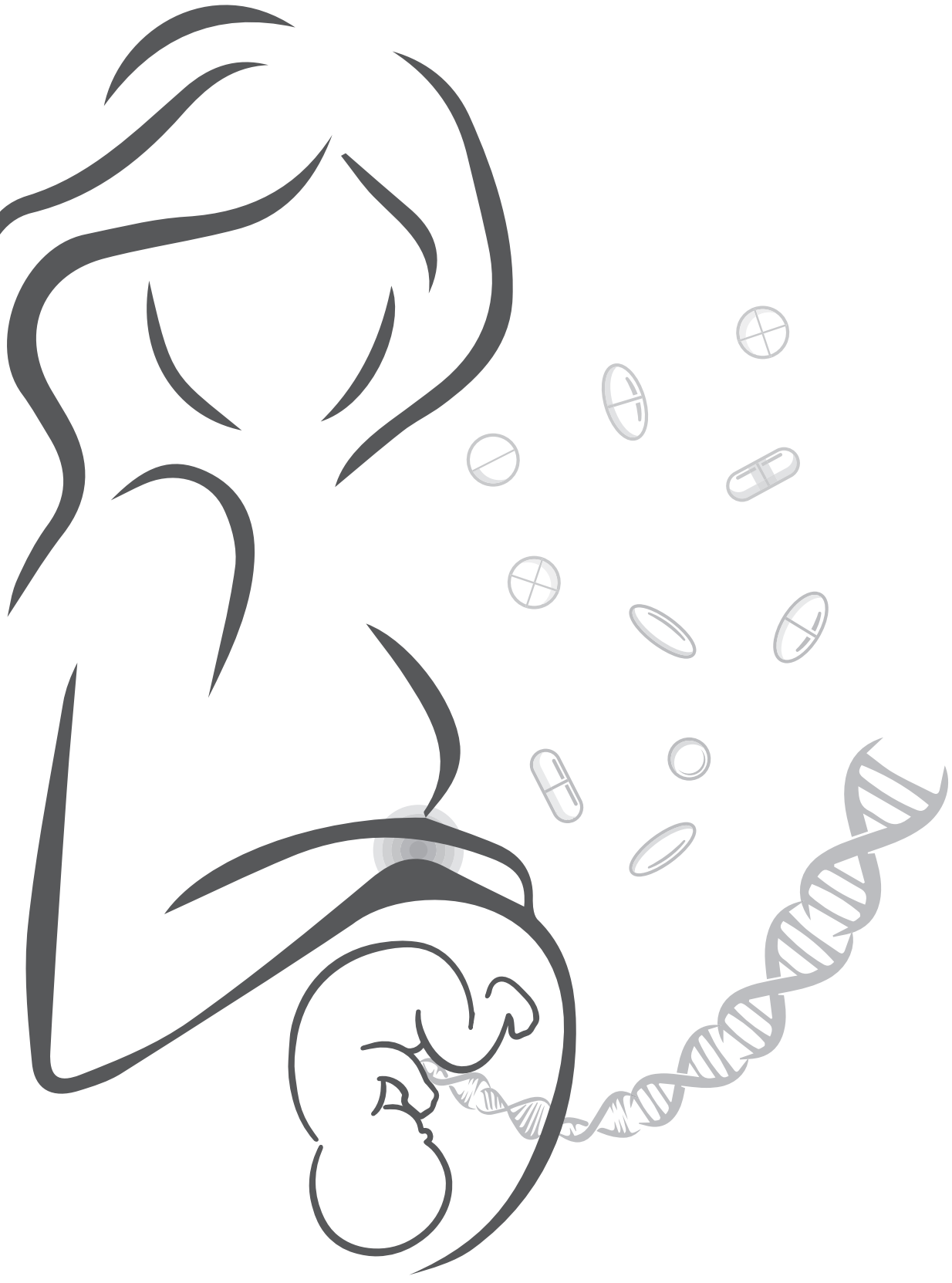
Hoofstuk 7

Dit hoofdstuk focust op het geven van borstvoeding in vrouwen met RA. De World Health Organisation (WHO) adviseert moeders om hun kind minimaal 6 maanden uitsluitend borstvoeding te geven en borstvoeding te blijven geven tot minimaal het 2e levensjaar. Borstvoeding heeft vele voordelen voor zowel moeder als kind. Moedermelk is het optimale voedingsmiddel voor zuigelingen en het versterkt de band tussen moeder en kind. Daarnaast heeft moedermelk meerdere immunologische en metabole voordelen voor het kind. Deze voordelen zijn het grootst bij kinderen met een laag geboortegewicht. Bij moeders met RA worden baby's vaker met een lager gewicht geboren, dus in deze groep zou borstvoeding een toegevoegde waarde kunnen hebben. Uit hoofdstuk 7 blijkt dat moeders met RA minder vaak beginnen met het geven van borstvoeding aan hun kind. Bovendien geven zij minder lang borstvoeding dan vrouwen uit de algemene populatie. Eén maand na de bevalling geeft 43% van de vrouwen met RA borstvoeding tegenover 63% van de vrouwen uit de algemene populatie. Na 3 maanden geeft 26% van de moeders met RA nog borstvoeding versus 46% van de moeders uit de algemene bevolking. Na 6 maanden is dit gedaald tot 9% van de moeders met RA versus 41% van de moeders uit de algemene bevolking. Een andere belangrijke bevinding is dat ongeveer 60% van de moeders met RA aangeeft dat ze zijn gestopt met het geven van borstvoeding om te kunnen starten met het gebruik van RA medicatie. Echter, bleek dat bijna de helft van deze vrouwen onnodig de borstvoeding had gestaakt, omdat de medicatie die zij herstartten veilig wordt geacht tijdens het geven van borstvoeding. Andere mogelijke redenen van staken van borstvoeding, zoals "geen zin", "niet genoeg melk", "moeite met aanleggen" zijn vergelijkbaar met de algemene populatie.

Gezien de voordelen van borstvoeding is er dus gezondheidswinst te boeken door artsen, verpleegkundigen en door patiënten meer educatie aan te bieden ten aanzien van het geven van borstvoeding in combinatie met het gebruik van medicatie.

Hoofdstuk 8

Tot slot worden in dit hoofdstuk, de algemene discussie, de nieuwe inzichten gebaseerd op dit proefschrift en de klinische implicaties hiervan beschreven. Bovendien worden de krachten en beperkingen van de PARA, FEPRA en HAIR-studie besproken. Daarnaast wordt de generaliseerbaarheid van de bevindingen bediscussieerd. Dit hoofdstuk eindigt met de aanbevelingen toekomstig onderzoek welke gebaseerd zijn op de bevindingen uit dit proefschrift.



Addendum

AUTHORS AND AFFILIATIONS

Author	Affiliation
de Rijke Y.B.	Department of Clinical Chemistry, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands
Dolhain R.J.E.M.	Department of Rheumatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands
Duijts L.	The Generation R Study Group, department of Pediatrics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands
Felix J.F.	The Generation R study Group, department of Epidemiology, department of Pediatrics, Erasmus MC, University Medical Center, Rotterdam, the Netherlands
Hazes J.M.W.	Department of Rheumatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands
Mandaviya P.R.	Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands
van den Akker E.L.T	Department of Pediatric Endocrinology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands
van Meurs J.B.J.	Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands
van Rossum E.F.C.	Department of Internal Medicine, Division of Endocrinology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands

LIST OF ABBREVIATIONS

11βHSD2	11beta-hydroxySteriod dehydrogenase type II
ACPA	Anti-citrullinated protein antibody
ACR	American college of Rheumatology
AMH	Anti-mullerian hormone
Anti-CCP	Anti-cyclic citrullinated peptide
BCG	Bacillus Calmette-Guérin
BMI	Body mass index
CDAI	Clinical disease activity index
CI	Confidence interval
COX	Cyclooxygenase
CRP	C-reactive protein
CGI	Cytosine guanine islands
CpG	Cytosine-phosphor-guanine
DAS28	Disease activity score in 28 joints
DAS28-CRP(3)	Disease activity Score in 28 joints using C-reactive protein levels
DHEA	Dehydroepiandrosterone
DMARDs	Disease-modifying antirheumatic drugs
DNA	Deoxyribonucleic acid
DOHaD	Developmental origins of health and disease
DXA	Dual X-ray absorptiometry
EAM	Extra articular manifestation
ESR	Erythrocyte sedimentation rate
eQTM	Expression quantitative trait methylation
EULAR	European league against rheumatism
Fab	Fragment antigen binding
Fc	Fragment crystallizable
FEPRA	Fetal programming in rheumatoid arthritis
GlcNAc	N-acetylglucosamine
HAQ	Health assessment questionnaire
HAQ-DI	Health assessment questionnaire disability index
HCQ	Hydroxychloroquine
HLA	Human leukocyte antigen
HPA	Hypothalamic-pituitary-adrenal
IBD	Inflammatory bowel disease
IgG	Immunoglobulin G
IL	Interleukin
IL1Ra	Interleukin-1 receptorantagonist
IQR	Interquartile range
IUI	Intrauterine insemination
IVF	In vitro fertilization
LC-MS/MS	Liquid chromatography–tandem mass spectrometry
MBL	Mannose-binding lectin
MC	Medical center
MTX	Methotrexate
N	Number
NSAIDs	Nonsteroidal anti-inflammatory drugs
OR	Odds ratio
PARA	Pregnancy-induced amelioration of rheumatoid arthritis
PRN	Perinatal registry Netherlands
QC	Quality control
QQ	Quantile-quantile
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RNA	Ribonucleic acid
SD	Standard deviation
SDAI	Simplified disease activity index
SDS	Standard deviation score
SES	Socioeconomic status
SGA	Small-for-gestational-age
SIDS	Sudden infant death syndrome
SLE	Systemic lupus erythematosus
SSZ	Sulfasalazine
sTNFR	Soluble tumor necrosis factor receptors
Th	T helper
TNF	Tumor necrosis factor
Tregs	Regulatory T-cells
TTP	Time to pregnancy
VAS	Visual analogue scale
WHO	World health organization

ABOUT THE AUTHOR

Hilal Ince-Aşkan was born on the 10th of May 1988 in the Hague, the Netherlands. In 2006 she graduated from Gymnasium at the secondary school Segbroek College, the Hague.
After finishing secondary school, she started studying medicine at the Leiden University Medical Center. From 2010 to 2012 she passed through her internships in different hospitals. This period was finalized with a specialisation internship at the department of Internal Medicine at the Westeinde hospital, the Hague. Right after graduating with distinction from medical school in September 2012 Hilal started working as a resident at the department of Internal Medicine at the Westeinde hospital (supervisor dr. A.H. Bootsma). She worked there at the departments of general medicine, gastroenterology, oncology, nephrology, pulmonology, cardiology and at the emergency department.

In May 2013 Hilal was transferred to the Daniel den Hoed clinic, the Cancer Institution in Rotterdam. She worked here for almost a year as a resident at the department of Oncology (supervisor dr. E. van Meerten). In April 2014 Hilal started working as a PhD candidate at the department of Rheumatology of the Erasmus MC, under supervision of prof. dr. J.M.W. Hazes and dr. R.J.E.M. Dolhain. During her PhD, she successfully finished a master in Genetic Epidemiology at the Netherlands Institute for Health Sciences (NIHES), Rotterdam.

In January 2019 she started her specialization into Rheumatology at the Erasmus MC in Rotterdam (supervisor dr. R.J.E.M. Dolhain). As a part of her specialization, she will work the first two years at the department of internal medicine of the Maasstad Hospital in Rotterdam (supervisor dr. E. M.A. van den Dorpel).

Hilal married on the 30th of March 2013 with Sebati Ince. Three years later, on the 31st of March 2016, they became proud parents of their son Milhan.

LIST OF PUBLICATIONS

Ince-Aşkan H., Dolhain R.J.E.M. *Pregnancy and Rheumatoid Arthritis.*
Best Pract Res Clin Rheumatol. 2015 Aug-Dec;29:580-96.

Ince-Aşkan H., Hazes J.M.W., Dolhain R.J.E.M. *Is Disease Activity in Rheumatoid Arthritis during Pregnancy and after Delivery Predictive for Disease Activity in a Subsequent Pregnancy?*
J Rheumatol. 2016 Jan;43:22-5.

Ince-Aşkan H., Hazes J.M.W., Dolhain R.J.E.M. *Identifying clinical factors associated with low disease activity and remission of rheumatoid arthritis during pregnancy.*
Arthritis Care Res. (Hoboken) 2017; 69:1297-303.

Ince-Aşkan H., Hazes J.M.W., Dolhain R.J.E.M. *In rheumatoid arthritis fewer women breastfeed their offspring compared with women from the general population; results from a nationwide prospective cohort study.*
J Rheumatol. 2018 [accepted]

Ince-Aşkan H., Mandaviya P.R., Duijts L., Felix J.F., van Meurs J.B.J., Hazes J.M.W., Dolhain R.J.E.M. *Altered DNA methylation in children born to mothers with rheumatoid arthritis during pregnancy.*
2018 [submitted]

Ince-Aşkan H., van den Akker E.L.T., de Rijke Y.B., van Rossum E.F.C., Hazes J.M.W., Dolhain R.J.E.M. *Associations between antenatal prednisone exposure and long-term cortisol and cortisone concentrations in children born to women with rheumatoid arthritis; results from a nationwide prospective cohort study.*
RMD Open. 2018 [accepted]

PHD PORTFOLIO

Name	Hilal Ince-Aşkan
Erasmus MC Department	Rheumatology
Research School	Netherlands Institute for Health Sciences (NIHES)
PhD period	April 2014 – October 2018
Promotor	Prof. Dr. J.M.W. Hazes
Copromotor	Dr. R.J.E.M. Dolhain

	Year	Workload ECTS
General academic skills	**2014-2018**	**5.8 (total)**
Course Integrity in Research, Erasmus MC	2014	0.4
Website Editorial Course	2014	0.4
BROK ('Basiscursus Regelgeving Klinisch Onderzoek), Erasmus MC	2015	1.0
Biomedical English writing and communication	2018	4.0
Master of Genetic Epidemiology	**2014-2017**	**70 (total)**
Core curriculum:		
Study design	2014	4.3
Genetic-epidemiologic research methods	2014	5.1
Biostatistical methods I: Basic principles	2015	5.7
Biostatistical methods II: Classical regression models	2015	4.3
In-depth courses:		
Linux for scientists	2014	0.6
SNPs and human diseases	2014	1.4
Courses for the quantitative researcher	2015	1.4
Advances in genome-wide association studies	2015	1.4
Family based genetic analysis	2015	1.4
Analysis of next-generation sequencing data	2015	1.4
Erasmus Summer Programme:		
Principles of research in medicine	2014	0.7
Principles of genetic epidemiology	2014	0.7
Genomics in molecular medicine	2014	1.4
Masterclass: advances in genomics research	2014	0.4
Genome wide association analysis	2014	1.4
Health economics	2015	0.7
Primary and secondary prevention research	2015	0.7

	Year	Workload ECTS
Master of Genetic Epidemiology (Continued)	**2014-2017**	
Erasmus Summer Programme:		
Logistic regression	2015	1.4
Fundamentals of medical decision making	2015	0.7
Joint models for longitudinal and survival data	2015	0.7
Epigenetics	2017	0,7
Research period	2017	33.5
Seminars and Workshops	**2014-2018**	**3.0 (total)**
Weekly department research meetings (attendance & presentations)	2014-2018	1.0
PhD Days, Erasmus MC	2014-2018	1.0
Rheumatology Cicero meetings	2014-2018	1.0
(Inter)national Conferences	**2014-2018**	**5.5 (total)**
8th International Conference on Reproduction, Pregnancy and Rheumatic Diseases, Trondheim, Norway [*attendance*]	2014	0.5
The European League Against Rheumatism (EULAR) Conference, Rome, Italy [*poster presentation in tour*]	2015	1.0
Rheumatology Education and Learning (REAL) symposium, Amersfoort, The Netherlands [*oral presentation, invited speaker*]	2016	1.0
The European League Against Rheumatism (EULAR) Conference, Amsterdam, the Netherlands [*poster presentation*]	2018	1.0
10th International Conference on Reproduction, Pregnancy and Rheumatic Diseases, Bern, Switzerland [*3 oral presentations*]	2018	2.0
Teaching	**2014-2018**	**Total (5.0)**
Tutor 1st year medical students	2014 - 2016	2.0
Coach for bachelor medical students ("professional development")	2015 - 2017	3.0

DANKWOORD

Starting strong is good, finishing strong is epic - R. Sharma

Het einddoel is bereikt, mijn proefschrift is af! Met dit proefschrift kijk ik terug op 4 jaar hard werken, 18.000 kilometer autorijden door heel Nederland en vele ups en downs. Met grote passie begon ik aan dit project en met grote voldoening ben ik dit dankwoord aan het schrijven. Een promotietraject doorloop je niet alleen. Dit is dan ook het gedeelte van mijn proefschrift waarin ik iedereen die betrokken is geweest mag bedanken. Het schrijven van mijn dankwoord was bijna net zo moeilijk als het uitleggen aan familie en vrienden dat promoveren toch echt iets anders is dan studeren.

Feeling gratitude and not expressing it is like
wrapping a present and not giving it - W.A. Ward

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When you're in need of love they give you care and attention
Friends will be friends

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Would you help me, mother, father?
I've became so wisdom proof

Mijn ouders, Münevver en Ahmet Aşkan, annem ve babam, size ne kadar teşekkür etsem az gelir. Bedankt dat jullie mij altijd hebben gestimuleerd om meer te bereiken in het leven en hogerop te komen. Jullie geloofden er altijd in dat ik het ver zou schoppen en met jullie

hulp is dat ook gelukt. Bedankt voor alle mogelijkheden die jullie mij hebben geboden en alle offers die jullie hebben gebracht. Jullie mogen heel trots zijn op jullie zelf, nog trotser dan dat jullie op mij zijn! Het is daarom ook vanzelfsprekend dat ik mijn proefschrift aan jullie heb opgedragen.

Could you help me, darling sister?
When I'm stuck and all alone

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And when I'm losing all my energy
You're like my only working remedy

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Your little hands wrapped around my finger
And it's so quiet in the world tonight

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