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Genetic factors in human reproduction a trade off between procreation and longevity

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GENETIC FACTORS IN HUMAN REPRODUCTION

A trade off between procreation and longevity

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Cover photo: F.M. van Dunné (The hand of my 90 year old grandmother with the hand of my two-month-old son, September 2001)

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Genetic factors in human reproduction.
A trade off between procreation and longevity

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in 1968

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Overige leden:	Prof. dr. M.H. Breuning
	Prof dr. E. Briët

In memory of my grandfather, Prof. dr. J.J. Dozy 1909-2004

For Jan Job, Pieter, Mathijs and Elenora

CONTENTS

1

General introduction

GENERAL INTRODUCTION

Current biological thinking emphasizes that organisms are programmed for fitness, maximizing the probability of transferring one's genes to the next generation. Fitness is the result of the organism being fertile and having the opportunity to raise its offspring to adulthood. This requires a sufficient investment in both reproduction and in maintenance of the body allowing the necessary post-reproductive survival. It is therefore plausible that genes that regulate fertility are interrelated with those regulating life span. Whether there are the same genes influencing both reproduction and longevity may also be possible. For instance, the insulin/IGF-1 pathway regulates both aging and reproduction, but it regulates the two processes independently of one another. Treating worms with *daf-2* RNAi from the time of hatching extends life span and delays reproduction, but treating them as young adults extends life span to the same extent with little or no effect on reproduction[1]. This is interesting because it hints at evolutionary flexibility: single mutations affecting this pathway could potentially affect both aging and reproduction or, alternatively, one but not the other[2].

1. REGULATING HUMAN LIFESPAN

From an evolutionary point of view there is no need for a perfect human body. Irrespective of the species studied, animals (including humans) that live in their natural habitat do not grow old because of the high risk of mortality from environmental factors that is disease or predators. This reduces the probability of long-term survival. A perfect body, a prerequisite for immortality, therefore does not seem credible. By means of this evolutionary approach, the moment that the offspring have reached the reproductive age, the necessity to live any longer has gone.

It is a tantalizing question how our bodies manage to keep up as we continuously challenge the end of life—why is it that we still live longer? The reality is that our bodies continuously accumulate damage from wear and tear. As time goes by, the risk of mortality grows. Absence of ageing is thus only attainable if we have an unlimited ability to maintain and repair our bodies; an ability that prevents permanent damage from occurring and keeps our bodies in perfect condition. In 1977, Thomas Kirkwood took this idea a major step further and

proposed that investment in maintenance and repair comes at the cost of investment in reproduction[3]. His theory is of an illuminating simplicity. Too little investment in the maintenance and repair of our bodies will lead to premature death and a low probability of having progeny; our biological fitness will thus be low. On the other hand however, too much investment in maintenance and repair will lead to a decrease in reproductive success, as resources are not unlimited. Every species trades investments in maintenance and repair against investments in reproduction to optimize evolutionary fitness under the specific environmental conditions in which they live. The theory helps us to understand why species that suffer high mortality from their environment invest a great deal in reproduction to prevent extinction, whereas species under less environmental pressure invest more in maintenance and repair and live longer—although at the cost of reproductive success.

The past two decades have brought experimental evidence for this trade-off, also known as the 'disposable soma theory'. A major methodological problem in studying human reproduction in relation to lifespan is the fact that specific environmental conditions determine the number of offspring and better survival, causing spurious correlations. Environmental conditions which affect early development of individuals, such as the quality and quantity of nutrition received in utero and infancy, predict the onset of many chronic diseases in adulthood, affect longevity and may also influence a range of measures of reproductive performance in human populations. These associations are proposed to result from fetal programming, where a stimulus or insult during a critical period early in life may permanently affect body structure, physiology, and metabolism[4]. Therefore, instead of adjusting for differences in socio-economic class, Westendorp & Kirkwood relied on the genealogies of the British aristocracy that for centuries embodied the upper crust of society[5]. This produced a unique, uniform population sample for which environmental conditions were equal within a certain time frame. In their study the age of death of the aristocratic women was plotted against the number of children that they had. The number of children was found to be small when women had died at an early age, increased with age at death, reaching a plateau through the sixth, seventh and eighth decades of life. However for women who died at ages of 80 years and over the number of children decreased again. In line with the disposable soma theory, women who reached very old age had significantly fewer children than those

who died at middle age. Apparently, women whose bodies had better durability due to greater investment in maintenance and repair lived longer, but at the cost of reproductive success.

It is known that variation in lifespan is in part the result of an individual ability to avoid or cope with internal and external damage, which has a strong genetic basis[6]. For example, single point mutations in the more than 17,000 genes of the worm *C. elegans* can lower the rate of aging and lengthen life span up to nearly five times as long as the wild-type worms[7]. In mice a single point mutation in the p66shc gene delays the rate of aging and extends average life span by about 30% [8]. These experimental data suggest that the majority of age-related changes are under coordinated genetic control[9]. Several observational studies in humans have also explored the genetic component in susceptibility to death. During the last decade a number of twin studies has shown that approximately 25% in the variation of human lifespan is explained by genetic factors[10;11]. The remainder of the variation has to be explained by private environmental factors and gene-environment interaction. Moreover, recent studies have demonstrated a clustering of extreme longevity within families[12;13].

Taken together, genetic factors play an important role in the regulation of human life span but the exact pathways remain to be elucidated. It is an intriguing idea that these pathways are interrelated with the regulation of human reproduction. Here we take the view that the chance of identifying the critical genes in either or both of these characteristics is likely to be increased when studying both characteristics at the same time.

2. HUMAN REPRODUCTION

Human reproduction is a process that appears remarkably inefficient. During each cycle about 20 ovarian follicles are triggered to start the process of maturation, usually only one completes this process and is ovulated. This is followed by an average probability of conceiving of about 20% per cycle[14]. Only about 30-50% of all conceptions result in a live birth, most will be lost even before the next menstrual date[15;16] (Figure 1 and 3). Nevertheless this inefficient process produces very good outcomes as the vast majority of ongoing pregnancies will result in the birth of a healthy child, who will eventually pass their genes on

to the next generation[17]. A longer time to pregnancy (fecundity) and miscarriages is an inevitable by-product of such a process.

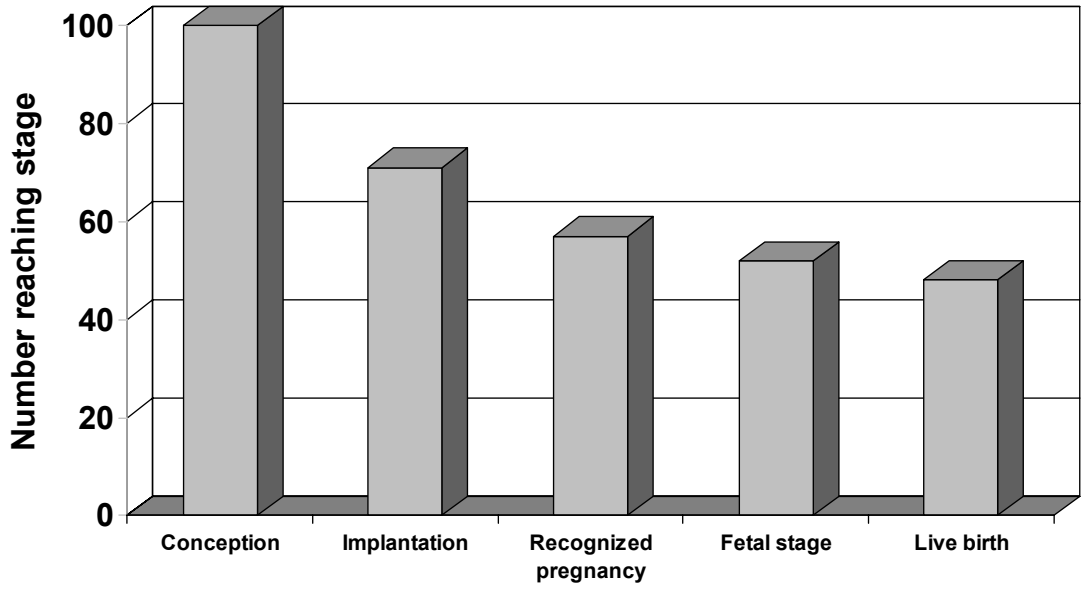

Figure 1. The fate of a fertilised ovum is a poor one[18].

2.1 FECUNDITY

In general there are 6 days in an average woman's menstrual cycle that intercourse can result in a pregnancy; these 5 days before ovulation and the day of ovulation are jointly referred to as the 'fertile window'[19-21] (Figure 2).

Fecundity is defined as the capacity for producing offspring, or the probability of a couple conceiving in a menstrual cycle. It can be measured by assessing the time period taken to conceive (time to pregnancy). Fecundity is influenced by a great number of factors like frequency of intercourse and regularity of menstrual cycle[22], sperm count[23], maternal age[19], body mass index[24] and recent use of oral contraceptives[25]. Also a negative lifestyle (i.e. smoking, alcohol, tea/coffee consumption) is dose dependently associated with a reduction in fecundity[26].

Overall the average fecundity rate per cycle in humans is about 15-20%[27] with a maximum of 30-40%, which is achieved only in the first few cycles[28] including non-viable pregnancies. Roughly 55-65% couples will achieve a pregnancy within the first 3 cycles and 80-90% in the first 12 cycles. Although the likelihood of a spontaneous pregnancy decreases with the duration of unexplained sub-fertility[27], given time, most couples will eventually conceive naturally. Ultimately 3-5% couples will result with definite infertility (inability to conceive)[29].

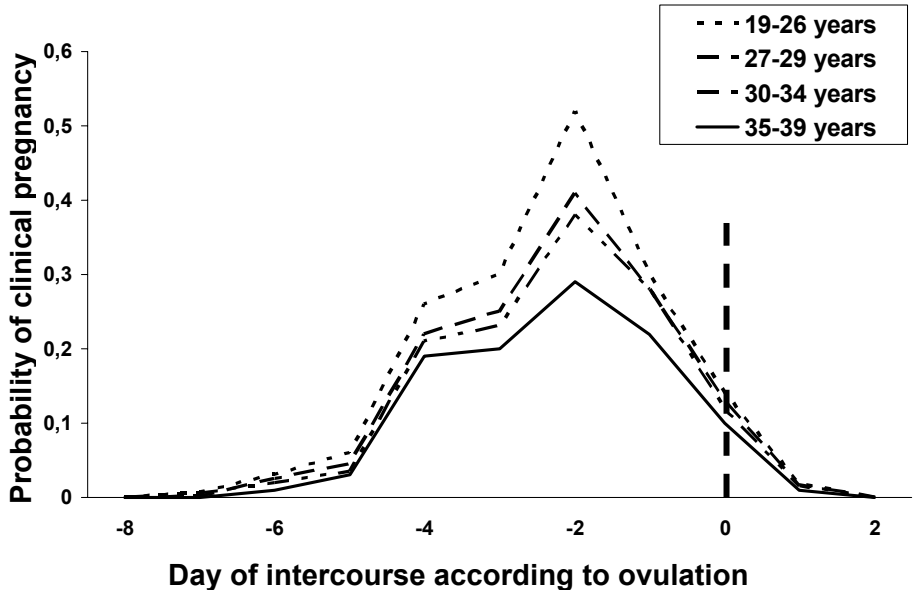

Figure 2. Probability of clinical pregnancy following intercourse on a given day relative to ovulation (day 0) for women of average fertility aged 19–26, 27–29, 30–34 and 35–39 years (European Study of Daily Fecundability, 433 pregnancies), adjusted for male partner's age[19].

2.2 MISCARRIAGE

A miscarriage is the premature expulsion of a nonviable fetus from the uterus, usually before the middle of the second trimester of gestation; it is also referred to as spontaneous abortion.

Once a pregnancy has been established there is a risk of miscarrying. Only 30-50% of all conceptions result in the birth of a child[15] (figure 3). Most pregnancies fail even before the next menstrual date is due and the woman in question is not yet aware of the pregnancy. This biochemical pre-clinical pregnancy will end around the time of the expected menstruation and

will appear like a normal cycle without fertilisation[30]. Of the recognized (clinical) pregnancies 10-15% will end in a miscarriage[14]. Of these clinical miscarriages about 90-95% will occur before fetal cardiac activity has been detected (embryo loss) and only 2-5% occur thereafter[31-33].

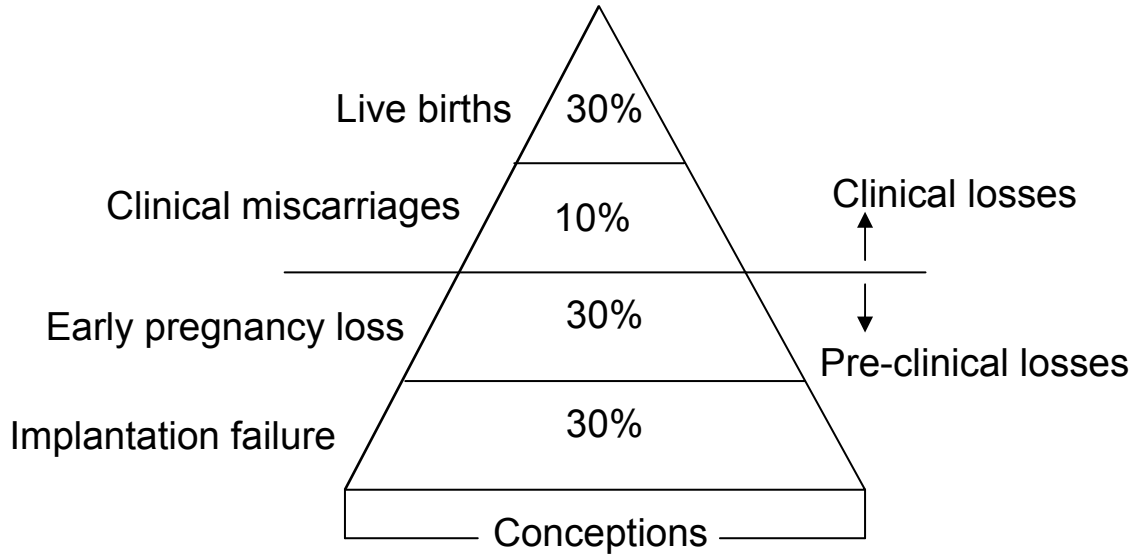

Figure 3. The pregnancy loss Iceberg; an overview of the outcome of spontaneous human pregnancy. A total of 70% of conceptions are lost prior to live birth. The majority of these losses are prior to the time of the missed menstruation and are not noticed. Adapted from Macklon 2002[15].

The most likely cause of miscarriage is the formation of an abnormal embryo or fetus. Miscarriages may therefore be seen as a safety mechanism of Mother Nature, preventing a severely abnormal human being to be formed. A chromosomal abnormality in the conceptus is the most frequent error leading to a miscarriage, accounting for 50-80% of all miscarriages[15;34;35]. A morphological abnormality of the fetus without a chromosomal aberration is the cause of fetal demise in 15-18% of miscarriages[35].

Other general etiological categories of miscarriages are thought to include immunologic disorders (anti-phospholipid syndrome, anti-cardiolipin antibodies and lupus anticoagulant), thrombotic disorders (factor V Leiden mutation, prothrombine G20210A mutation, deficiencies in -protein C, -protein S and -antithrombin III), uterine pathology, endocrine dysfunction, and environmental factors[36;37]. Infectious diseases, malnutrition, chemical

exposures, (illegitimate) drugs, alcohol- and nicotine abuse have all been named as increasing the chance of a miscarriage[38]. Furthermore there is a growing risk of miscarriage with an increasing maternal age[39] (figure 4). At 42 years of age more than half of all clinically recognized pregnancies end in a miscarriage or fetal loss[40].

As miscarriages occur regularly, three consecutive miscarriages (recurrent miscarriage) will occur in 1-3% of all fertile couples[41], higher than the expected rate of 0.3%. In about 50% of couples experiencing recurrent miscarriages a probable cause cannot be found. A genetic predominance or an innate mechanism in couples suffering (recurrent) miscarriages therefore seems feasible.

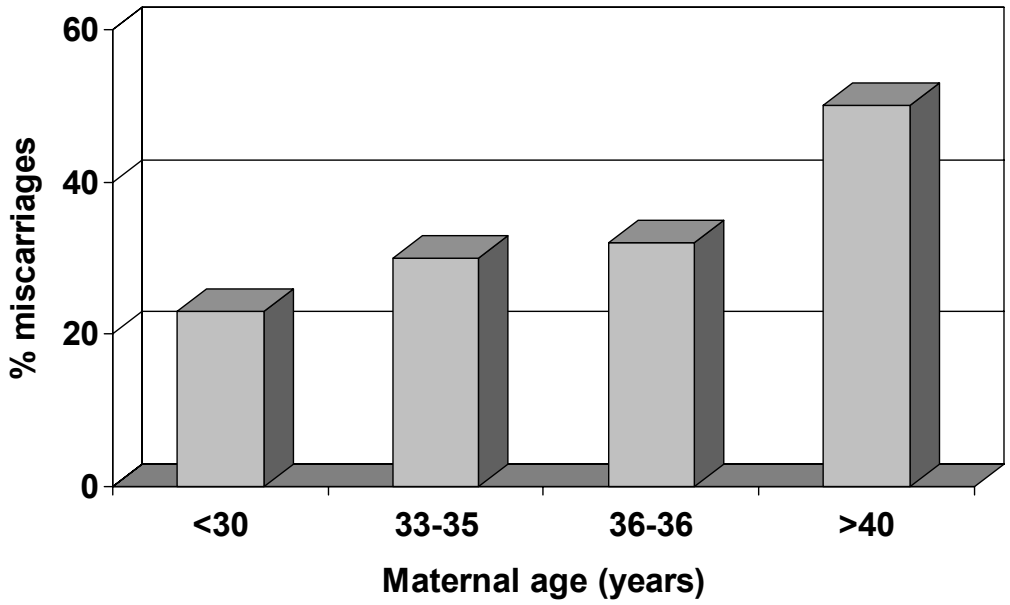

Figure 4. Influence of maternal age on outcome of subsequent pregnancy. After Clifford 1997[39].

3. IMMUNOLOGY

The maternal immunologic response to the fetus needs to be appropriate for successful implantation and development of the pregnancy[42] without suffering the state of general immunity. When the immune system mistakes 'self' tissues for 'non-self' and mounts an inappropriate attack, it can result in an autoimmune disease. Rheumatoid arthritis (RA) and

systemic lupus erythematosis (SLE) are examples of autoimmune diseases that predominantly occur in females. During the reproductive years these autoimmune diseases can influence the outcome of pregnancy and vice versa, pregnancy will influence the disease[43;44]. SLE and RA react differently in pregnancy; pregnancy induces improvement or even remission of disease activity in 75% of RA patients[45], whereas SLE tends to flare during pregnancy in about 50% of patients[46]. Women with SLE have a higher risk of pregnancy complications like for instance miscarriages, premature birth, small for gestational age and pre-eclampsia[47]. These pregnancy complications are related to the presence of various auto-antibodies (antiphospholipid antibodies, lupus anti coagulans, anti cardiolipin antibodies) but will increase even more with a high SLE disease activity[48]. A high disease activity is associated with an increase in cytokine production. Cytokines are important mediators in autoimmune diseases like SLE and RA and they are also thought to play an important role in the acceptance and maintenance of pregnancy[44]. The different reaction of these autoimmune diseases to pregnancy may be explained by an altered cytokine production. In the general population pregnancy outcomes per se may be influenced by variations in cytokine production and therefore influence pregnancy failure.

3.1 CYTOKINES

Cytokines are soluble proteins produced by various cells such as activated lymphocytes and macrophages. They are involved in the control of local and systemic responses of the immune system. No cytokine has a unique effect and the action of one cytokine may overlap that of another. Roughly, they can be divided into Th-1 and Th-2 cytokines and it is assumed this immune response is in balance. Traditionally this division into Th-1 and Th-2 categories has been dependent upon the immune cell of origin and the immunological effects that they bring about. Th-1 cells are the main effectors of cell-mediated immune responses and produce Th-1 cytokines that mainly have a pro-inflammatory effect, this is important for protection against infections[49]. Th-2 cells are the main effectors of antibody-mediated humoral responses and produce Th-2 cytokines that primarily have an anti-inflammatory effect and down regulate the pro-inflammatory response. An example of a Th-1 cytokine is Tumor Necrosis Factor α (TNFα) and for a Th-2 cytokine Interleukin-10 (IL-10) is an example.

Enhanced secretion of anti-inflammatory Th-2 cytokines is a characteristic of a normal physiologic pregnancy[42]. This response is considered necessary for the acceptance of the semi-allogenic fetus[42;50;51]. Of the Th-2 cytokines, IL-10 is probably of particular importance. In a mouse model deficient for anti-inflammatory cytokines, the mice experienced elevated levels of fetal loss. Administration of anti-IL-10 further increased the fetal loss whereas administration of IL-10 reduced fetal loss significantly[52]. In humans fertilised ovum harvested by in vitro fertilisation (IVF) have been shown to induce IL-10 production in human lymphocytes[53]. In decidual cells of women with unexplained recurrent miscarriages a decreased production of Th-2 cytokines, including IL-10, was found compared to decidual cells of normal developing pregnancies[54]. Serum IL-10 levels are also reported to be low in pre-eclampsia[55].

As mentioned previously, cell mediated autoimmune diseases such as RA, are ameliorated during human pregnancy, while antibody-mediated diseases such as SLE are aggravated[43;44]. This indicates a weakening of the cell-mediated response and an enhancement of the antibody response, which also correlates with a down regulation of Th1-type activity and an enhancement of Th2-type activity. IL-10 seems to play a central role in the pathogenesis and disease flare induction of SLE[52], by contrast in RA there is a deficient Th-2 production lacking in IL-10[56]. The immunosuppressive effects of IL-10 are diverse (figure 5).

IL-10 has an immunosuppressive effect on T cells, monocytes, and macrophages by inhibiting release of pro-inflammatory cytokines[57]. Furthermore, IL-10 enhances B cell survival, proliferation, differentiation, and antibody production, and so effecting various autoimmune diseases[58]. Simplified, an increased IL-10 cytokine production may be an explanation for the remission of RA in pregnancy and increased flare probability in SLE in pregnancy. An innate predominance for IL-10 production in relation to fertility, fecundity and miscarriages seems plausible.

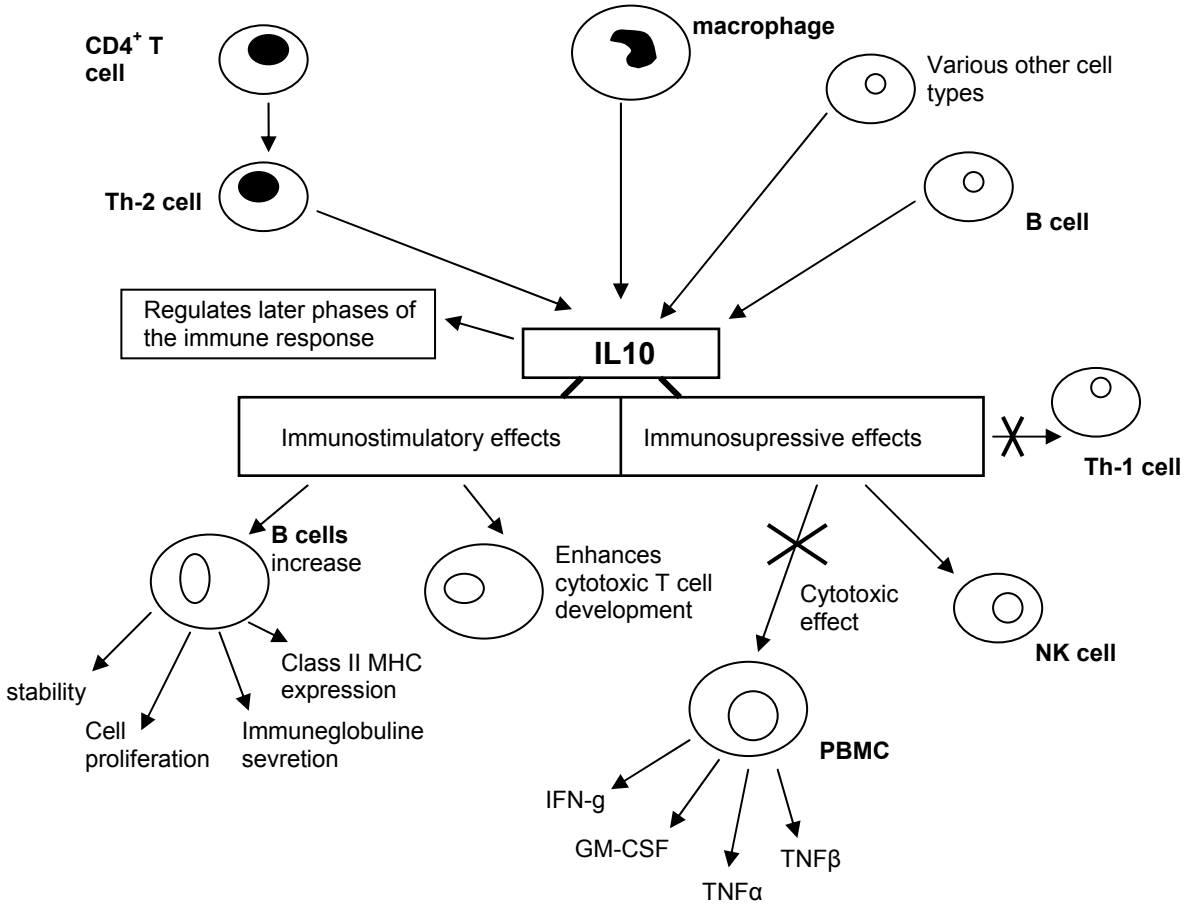

Figure 5. A simplified schematic figure of the IL-10 mechanism. Activation of various cells produce IL-10, IL-10 then plays an important immunostimulatory as well as inhibitory role. The inhibitory effect is indicated by 'X'.After Conti et al, 2003[59].

4. GENETICS

4.1 IL-10 POLYMORPHISMS

The production of cytokines is influenced by genetic factors. The human IL-10 gene is located on chromosome 1 and is composed of 5 exons[57]. IL-10 is highly polymorphic and at the promoter region several single nucleotide polymorphisms have been described. Single nucleotide polymorphism or SNP (pronounced 'snip') is a small genetic change, or variation, that can occur within a person's DNA sequence. The genetic code is specified by the four nucleotide 'letters' A (adenine), C (cytosine), T (thymine), and G (guanine). SNP variation occurs when a single nucleotide, such as an A, replaces one of the other three nucleotide

letters—C, G, or T. For example a SNP might change the DNA sequence A**A**GGCTAA to A**T**GGCTAA. SNPs occur in about 1 every 1000-2000 nucleotides[60].

Of the variation in IL-10 production 75% is genetically determined, indicated by monozygotic twin research[49]. The different SNPs in the IL-10 gene may explain the discrepancy in heritable IL-10 production capacity[61;62]. The IL-10 –1082A allele has been reported to be associated with an increase in IL-10 production in peripheral blood[56]. A correlation between IL-10 polymorphisms and various aspects of human reproduction remain to be clarified. However, an association between the IL-10 –1082GG genotype and recurrent miscarriages was found in a meta-analysis comprising 3 studies[63]. The exact interaction between IL-10 polymorphisms and longevity remains to be elucidated. An Italian study found that the *IL-10* gene SNP -1082G-A allele had a significant influence on the attainment of longevity in men[64] this in contrast to a Finnish population study where IL-10 promoter alleles and haplotype frequencies were not different between nonagenarians and controls[65]. These findings suggest that cytokine/longevity associations may have a population specific component, being affected by the population specific gene pool as well as by gene-environment interaction[64].

4.2 FACTOR V LEIDEN

The blood coagulation system is a complex cascade and can be divided in an intrinsic (contact phase) and extrinsic (tissue factor dependent) pathway. This system is tightly regulated. Several procoagulant, anticoagulant and fibrinolytic factors are involved. In 1994 in Leiden The Netherlands, Bertina first described a mutation involving an increased tendency in blood clotting[66]. This is a point mutation, or SNP, located on chromosome 1 (1q23) in the gene of factor V: a G→A transition in position 1691, in exon 10, that predicts the replacement of Arg 506 by Gln in the factor V molecule (factor V Leiden)[66]. The effect of the factor V Leiden mutation is that the activated factor Va produced cannot be inactivated completely by activated protein C (APC). In APC-resistance a higher tendency of blood clotting occurs increasing the risk of deep vein thrombosis (DVT) 7-fold[67]. Ninety-five percent of cases of APC-resistance are due to factor V Leiden mutation. Factor V Leiden is present in 3 to 10% of people of Caucasian origin[66;68]. Factor V Leiden incidence does not differ with age: in residents of 90 years and older a similar allele frequency was found compared to the general

population[69] In agreement to this, no age-related frequency decrease in the FVL 1691A allele was reported in a study conducted in the USA among 2689 voluntary blood donors ranging from 17 to 85 years old[70]. Although factor V Leiden mutation increases the risk of DVT in adult life, it therefore does not appear to influence the human lifespan overall[71]. However, it may influence human reproduction.

Pregnancy in general is a hypercoagulable state due to both a rise in certain coagulation factors and a fall in concentrations of anticoagulant proteins and fibrinolysis[72]. During pregnancy there is an increase in APC resistance, which will increase the chance of a thrombotic event, even more so in the presence of a factor V mutation. Due to this increase in thrombotic tendency it has been suggested that factor V Leiden mutation may be associated with various aspects of human reproduction such as (recurrent) miscarriage, pre-eclampsia, prematurity and small-for-gestational-age neonates[73-77]. However much controversy remains. The majority of women with a factor V Leiden mutation will experience an uneventful pregnancy with a normal outcome[78]. Furthermore, a positive effect of factor V Leiden on implantation has been postulated[79]. An improved implantation rate in intra-cytoplasmatic sperm injection (ICSI) pregnancies was reported if either the mother and/or the fetus carried the factor V Leiden mutation[80]. Possibly an increased local thrombotic tendency will increase the likelihood of implantation of a blastocyst (embryo).

5. OUTLINE OF THIS THESIS

The principal aim of this study is to assess the role of certain genetic factors in early pregnancy in humans. There are probably numerous genetic factors influencing human reproduction. This thesis will highlight IL-10 and factor V Leiden. They are thought to interfere with human reproduction in different ways; IL-10 via an anti-inflammatory pathway and factor V Leiden as a result of an increased coagulation at the site of embryo implantation.

In addition a correlation between the ability to reproduce and human longevity is evaluated. For IL-10, with its anti-inflammatory trait, it is probable that an effect at survival level in the long run exists. This genetic factor may enhance one (reproduction) at the cost of the other (longevity), also referred as the 'disposable soma theory'.

As mentioned earlier, human reproduction and longevity are probably linked; one possibility is by way of various cytokines. In **Chapter 2** both pro-inflammatory Th-1 (IL-10) and anti-inflammatory Th-2 (TNFα) cytokines are assessed in relation to reproduction and longevity, in an attempt to explain a trade-off between fertility and survival to old age.

In **Chapter 3** the interleukine-10 gene is assessed on a genetic level. The innate IL-10 polymorphisms, SNPs, and their haplotypes are analysed in relation to fecundity and fertility in a cohort of subjects who have reached the age of 85 years.

An example of a pro-inflammatory Th-1 mediated disease is rheumatoid arthritis (RA) with a low innate IL-10 production. The hypothesis whether pregnancy failure (miscarriages and /or decreased fecundity, seen as non-Th-2 phenomenon) interferes with the progress of joint destruction in RA is investigated in patients seen at the Early Arthritis Clinic. The results are stated in **Chapter 4**.

A gene also thought to interfere with human reproduction is the factor V gene. Factor V Leiden mutation is a potentially harmful gene mutation that increases the chance of a deep vein thrombosis. The hypothesis of factor V Leiden increasing embryo implantation is investigated in **Chapter 5**, where female patients included in a large population-based case-control study (first time thrombotic event) were interviewed on their past reproductive history (fecundity and miscarriages).

As it is hypothesized that factor V Leiden increases embryo implantation one would expect that subjects with this mutation may have more children and have them within a shorter interval after their marriage. This may be best observed in a population lacking accessible contraception. In **Chapter 6,** in both males and females, the effect of factor V Leiden mutation on fecundity and fertility is analysed in a large cohort of people who have reached the grand age of 85 years.

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2

Optimizing human fertility and survival

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Whenever a gene for fertility is identified[1], it is bewildering to think how variants associated with impaired fertility could have spread so widely in the population despite their obvious fitness disadvantage. Impaired fertility presently affects about 1 in 7 heterosexual couples in developed countries[2]. To explain this paradox, we refer to evolutionary theories on longevity that assume costs of reproductive success[3]. Here we present an immunogenetic explanation for how evolutionary fitness optimizes selection for fertility with selection for survival.

Reproductive success is dependent on a Th2/Tr1 immune response at the fetal-maternal interface permitting pregnancy to proceed[4]. When we studied cytokine responsiveness of the innate immune system, we found that women of normal fecundity exhibit a cytokine profile that drives naive T-cells towards a Th2/Tr1 phenotype (Table 1). The probability of normal fecundity increased more than 10-fold when the innate cytokine profile of the women was characterized by high interleukin (IL)-10 and low tumor necrosis factor (TNF)-α responsiveness. The cytokine profile of women with impaired fertility was characterized by low IL-10 and high TNF-α responsiveness.

Table 1. Association between reproductive success and innate cytokine responsiveness

Production of IL-10	**low**	**Low**	**high**	**high**
Production of TNF-α	**high**	**low**	**high**	**low**
Women with normal fecundity (no)	1	11	14	6
Women with impaired fertility (no)	8	13	10	3
Odds ratio (95% CI)	1 (-)	6.7 (0.7-63)	11.2 (1.2-104)	16 (1.3-195)

Production of IL-10 (supportive of Th2/Tr1-cell development) and TNF-α (supportive of Th1-cell development) was determined in whole blood samples upon stimulation with endotoxin (1000 ng/ml) for 24 and 4 h, respectively. The concentrations of cytokines were measured in supernatants by ELISA and dichotomized as low and high around the median. Impaired fertility was defined as having had at least 3 consecutive spontaneous abortions before 16 weeks of gestation. Women with chromosomal abnormalities, anatomical uterine defects, pro-thrombotic disorders, and co-morbid conditions were excluded. CI: confidence interval.

Previous attempts to identify genetic polymorphisms that associate with different TNF-α responsiveness have been unsuccessful. Earlier we have identified haplotypes that segregate

with IL-10 responsiveness[5], as well as several novel single-nucleotide polymorphisms in the distal promoter of the gene encoding IL-10 (ref. 6). Here we present data on cytokine responsiveness in healthy subjects stratified for a nucleotide polymorphism at position −2849 of the IL-10 promoter (Fig. 1). It seems that carriers of the -2849 AA genotype have significantly lower IL-10 responsiveness upon stimulation with endotoxin.

Therefore, using a large set of married women with completed families[7], we tested whether this genotype was enriched among women with impaired fertility. Congruent with a low IL-10 phenotype as described, the -2849 AA genotype was two-fold more prevalent among 73 women who remained childless when compared to the prevalence among 323 women with normal fecundity (13.6% and 5.6% respectively, $\chi^2 = 5.9$, $P = 0.014$). This difference in genotype distribution strongly supports the notion that human fertility is dependent on the cytokine profile of the innate immune system.

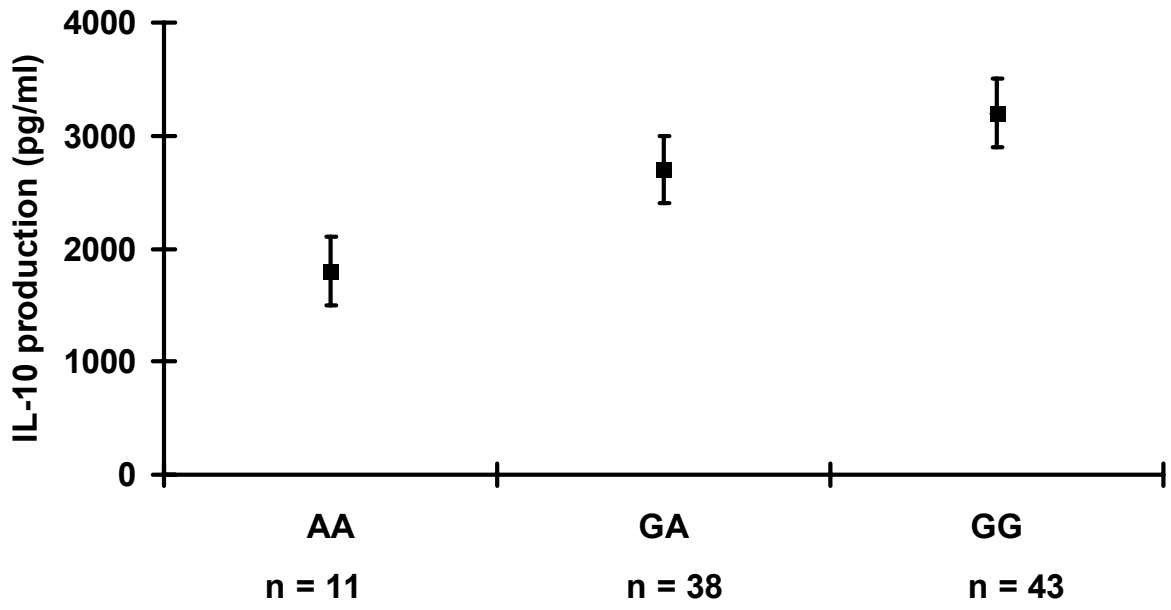

Figure 1. Data are presented as means ±s.e.m. Production of IL-10 was determined in whole-blood samples upon stimulation with endotoxin (1000 ng/ml) for 24 h. Sampling was performed in 92 unrelated healthy subjects and described earlier[8]. The single AG nucleotide polymorphism at position −2849 of the IL-10 promoter region 5' to the ATG start site was determined in genomic DNA[6]. IL-10 production was significantly lower in carriers of the AA genotype compared to the other genotypes, ANOVA: $P = 0.021$.

Critical for understanding the high proportion of women with impaired fertility is the relation of the innate immune system to the outcome of infectious disease. We have previously shown that subjects with the low IL-10 and high TNF-α responsiveness that drives naive T-cells towards a Th1 phenotype were protected from fatal outcome of infection[8]. In times when half of all newborns died from infection before reaching adolescence, survivors were strongly selected for resistance to infection. We propose that during evolution selection for fertility (a cytokine profile that favours the development of Th2/Tr1-type T-cells) is optimized with selection for survival (a cytokine profile that favours the development of Th1-type T-cells). The current data also provide an immunogenetic explanation for the inverse association between family size and longevity of British aristocrats[3] under conditions in which infection was a major cause of early death. Under those conditions, an innate cytokine profile supportive of Th1-type T-cells favoured survival of infectious diseases, including cholera and tuberculosis. However, women with such a cytokine profile would have been less likely to have successful pregnancies.

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3

Interleukin-10 promoter polymorphisms in male and female fertility and fecundity

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Abstract

Interleukin-10 (IL10) is assumed beneficial for a successful pregnancy; it may increase fertility and fecundity. Different IL10 promoter polymorphisms were analysed in association with fertility and fecundity in male and female subjects. From 1986 to 1999, all inhabitants of Leiden, The Netherlands reaching the age of 85 years were enrolled in the Leiden 85-Plus Study. Allele frequencies of IL10 polymorphisms at position –2849, -1082 and –592 were analysed in these subjects. The Registry of Births, Deaths and Marriages Leiden provided the dates of birth, marriage and birth(s) of children. Fertility was decreased in association with the –2849 A allele in females; 27% of the AA genotype carriers remained childless compared to 14% of the G allele carriers (OR: 2.2, 95% CI: 1.2-4.2, p=0.01). Effective fecundability was decreased in association with the –2849 A allele in females; 7% of female -2849AA genotype carriers had a child within 371 days of marriage (therefore conceived within 3 months of marriage) compared to 28% of female G allele carriers (OR: 0.2, 95% CI: 0.04-0.7, p=0.01). This suggests that the IL10 –2849 AA genotype is associated with a decreased fertility and fecundity in females; in male subjects no such association was observed.

Introduction

Interleukin-10 (IL10) is a multifunctional anti-inflammatory cytokine that is produced by various cells including monocytes, macrophages, B cells, T cells and mast cells(1;2). IL10 in return modulates the performance of these various cells with important consequences to their ability to activate and sustain immune and inflammatory responses(3). The role IL10 plays in the immune response is in inhibiting the production of various pro-inflammatory cytokines produced by a large number of different cells(2). There is large variation in IL10 production capacity between healthy individuals. These interindividual differences in IL10 production are largely under genetic control; 50-75% of the variation can be explained by genetic factors as demonstrated in twin studies(4-6). In the IL10 promoter region various single nucleotide polymorphisms (SNPs) have been described, in both the distal and proximal promoter region.

The effect of IL10 promoter polymorphisms on IL10 production has not been fully elucidated. The -2849AA genotype has been associated with significantly lower IL10 production upon endotoxin stimulation compared to the G genotype carriers(7). For the IL10 –1082 A allele it seems less clear; both a decreased IL10 production has been described related to the AA genotype(8;9), as well as an increased production, as well as no association(7;10). The IL10–592CC genotype has been related to a low IL10 production capacity after stimulation with S. pneumonia(10).

In pregnancy IL10 is considered one of the major immunoregulatory cytokines important for a successful outcome. Numerous studies have described that at the maternal-fetal interface IL10 production is increased. Moreover, decreased production of IL10 is associated with pregnancy loss and increase in pre-eclampsia(11). The IL10 polymorphisms at positions –2849, –1082 and –592 have been associated with a range of pregnancy-associated phenomena like recurrent miscarriages(12), preterm birth(13) and pre-eclampsia(14). The evidence for the -2849 polymorphism appears to be strongest associated with fertility. The IL10 -2849AA genotype was reported to be two-fold more prevalent among 73 married women who remained childless (RR 2.1, 95% CI 1.2-3.6), which was sustained in a similar study(15). The assumption was made that this genotype may reduce the chance of a successful pregnancy due to a decreased innate IL10 responsiveness.

The current study was initiated to further specify the role of IL10 in relation to two aspects of human reproduction; the ability to have a child (fertility) and the probability of a couple conceiving in a specific period of time (fecundity). This was assessed in a large cohort of subjects born in the late 19th and early 20th century, who were in their childbearing age in a time where modern contraceptive methods were unavailable. The present study is a continuation of earlier studies(15) with the distinction that not only more SNPs were analysed with their respective haplotypes, but also additional specified information on the subjects was obtained. Therefore, the data of both female and male subjects was analysed in relation to fertility, fecundity and the various IL10 polymorphisms.

MATERIALS AND METHODS

Subject recruitment

The Leiden 85-plus Study consists of two separate cohorts. A detailed description of both cohorts has been presented elsewhere(15;16). In short, subjects of the first cohort were enrolled between December 1986 and March 1989. During that period a total of 977 inhabitants of Leiden, The Netherlands, who were aged 85 and over were included. A second cohort of 85-year-old subjects, consisting of 599 subjects, was enrolled between September 1997 and September 1999. There were no selection criteria for health or demographics in either cohort. Of all 1576 subjects a blood sample was obtained. DNA was available for an unselected sample of 1278 subjects. The Leiden University Medical Centre medical ethical committee approved the study protocol for both cohorts.

Date retrievals

The Registry of Births, Deaths, and Marriages of the municipality of Leiden and the Central Bureau of Genealogy (CBG), The Netherlands, provided the date of birth, date of marriage(s), and birth dates of children of all study participants. The CBG is the major documentation and information center for family history and heraldry in The Netherlands. For 42 subjects there was insufficient information available on their marital history or their number or dates of birth of progeny. Hence, complete information was available for 1236 subjects.

Calculation of fecundity

Fecundity was defined as the calculated time interval between the date of (first) marriage and the date of birth of the first-born child. This concept of delay from marriage to the first birth has previously been defined as 'effective fecundability'(17). If the conception had taken place within the first 3 months of marriage, it can be assumed that these children were most likely born within 371 days of the marriage date. This was calculated by adding 3 months (91 days) to the median duration of a term pregnancy (280 days). Subjects with their first child born before marriage were excluded from analysis.

IL10 promoter gene polymorphisms

Participants were genotyped for the IL10 promoter gene at positions –2849, –1082 and –592. The typing of the IL10 G-2849A (rs6703630) polymorphism has been described previously(15). In short, genotypes were obtained using an Assay-by-Design (Applied Biosystems), consisting of PCR primers and TaqMan MGB probes. Amplification reactions were made at standard conditions. Real time PCR was performed on ABI 7900 HT (Applied Biosystems). The IL10 G-1028A (rs1800896) and IL10 C-592A (rs1800872) polymorphisms were genotyped by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (MS), using the Sequenom MassARRAYtm (Sequenom Inc.) methodology. Amplification reactions and parameters were based on the manufacturer's instructions.

Data analysis

Non-normally distributed data are presented as geometric means and 95% confidence intervals (CI). Differences in prevalence were compared by the Pearson Chi-square test with Fisher's exact test applied when at least one expected frequency was below 5. All tests were 2-tailed. Logistic regression models were applied to correct for age at marriage. P-values < 0.05 were considered statistically significant.

Alleles were considered to be in Hardy-Weinberg equilibrium if the observed genotype frequencies did not differ significantly (P<.05) from those expected when analysed by χ^2 test.

Odds ratios for haplotype comparisons were obtained from THESIAS (available from http://www.genecanvas.org). THESIAS is used for real data analysis, either for a binary, a quantitative or a survival outcome for haplotype-based association studies(18;19).

RESULTS

Baseline characteristics

For 1236 subjects complete information was available; however not all subjects had information for all three SNPs analysed. For 1185 (96%) subjects the IL10 –2849 status was known, for 1132 (92%) subjects the IL10 -1082 status was known and for 1130 (91%) the IL10 –592 status was known. Of 1043 (84%) subjects there was information on all three SNPs available.

Table 1. The fertility characteristics of all 1116 married subjects (759 female and 357 male) of the total 1236 (857 female and 379 male) subjects included in the Leiden 85 Plus Study.

	Married females n = 759	Married males n = 357
Childless	117 (15)	52 (15)
Number of children	2.7 (2.2)	2.8 (2.2)
Age at marriage	25.7 (6.4)	27.3 (4.9)
Age at 1st birth	26.2 (4.5)	28 (5.0)

Values are in n(%) or mean (SD).

Table I shows the general characteristics of all 1236 subjects included in this analysis. No significant differences were found. The cohort comprised of 857 women (69%) and 379 men (31%). The year of birth ranged from 1887 to 1914. Of the 857 female subjects 759 (89%) had been married at least once; of the 379 male subjects 357 (94%) had been married at least once. The year of birth of the first-born children ranged from 1910 to 1954 in female subjects and form 1912 to 1961 in male subjects. The age at marriage for the female subjects was comparable for the various IL10 genotypes analysed (data not shown). All IL10 SNPs were in Hardy-Weinberg equilibrium.

Table II. Association between IL10 genotype and fertility.

	Genotype*			OR (95% CI)		
	11	12	22	11 versus rest	22 versus rest	22 versus rest adjusted**
Females						
IL10 G-2849A						
Childless	50 (14)	46 (14)	14 (27)	0.9 (0.6-1.4)	2.2 (1.2-4.2)	2.3 (1.1-4.9)
≥ 1 Child	298 (86)	279 (86)	38 (73)			
IL10 G-1082A						
Childless	29 (16)	51 (14)	25 (17)	1.0 (0.6-1.7)	1.1 (0.7-1.9)	1.2 (0.7-2.0))
≥ 1 Child	153 (84)	308 (86)	126 (83)			
IL10 C-592A						
Childless	62 (15)	36 (15)	7 (23)	1.0 (0.6-1.5)	1.7 (0.7-4.0)	2.0 (0.8-5.3)
≥ 1 Child	352 (85)	208 (85)	24 (77)			
Males						
IL10 G-2849A						
Childless	24 (15)	24 (16)	3 (9)	1.1 (0.6-2.3)	0.5 (0.1-1.9)	0.4 (0.1-1.5)
≥ 1 Child	134 (85)	130 (84)	32 (91)			
IL10 G-1082A						
Childless	14 (17)	23 (14)	14 (18)	1.3 (0.6-2.6)	1.2 (0.6-2.3)	1.1 (0.5-2.2)
≥ 1 Child	64 (83)	147 (86)	69 (82)			
IL10 C-592A						
Childless	27 (14)	20 (17)	3 (19)	0.8 (0.4-1.4)	1.3 (0.4-4.8)	1.6 (0.4-6.2)
≥ 1 Child	171 (86)	97 (83)	13 (81)			

Values n (%), OR = Odds Ratio, 95% CI = 95% Confidence Interval.
*The -2849 genotypes are: 11 equals -2849 GG, 12 equals -2849 GA, 22 equals -2849 AA. The -1082 genotypes are: 11 equals -1082 GG, 12 equals -1082 GA, 22 equals -1082 AA. The -592 genotypes are: 11 equals -592 CC, 12 equals -592 CA, 22 equals -592 AA.** Adjusted by logistic regression for age at marriage

IL10 SNPs in association with fertility

Of the 759 married female subjects 117 (15%) of the marriages remained childless. For the 357 married male subjects this was 52 (15%). The total number of children was comparable for all SNPs analysed and was not related to the various SNP genotypes (data not shown). Fertility was classified according to whether the marriage remained childless or not; this was analysed per IL10 SNP at a genotype level and presented in Table II. Female –2849AA genotype carriers had a 2 fold higher likelihood of having a marriage that remained childless (Odds Ratio (OR) 2.2, 95% confidence interval (CI): 1.2-4.2). When adjusting for age at marriage the results remained similar (OR 2.3, 95% CI: 1.1-4.9). There was no such relation found for the other IL10 SNPs. No relation between the IL10 haplotypes and fertility could be found.

In male subjects no clear association between any of the IL10 SNPs and fertility (childlessness) was found. Males carrying the –2849AA genotype did not have a significantly different odds of remaining childless in marriage, (OR 0.5, 95% CI 0.1-1.9).

IL10 SNPs in association with fecundity

Effective fecundity is presented in Table III with the calculated conception time of married subjects analysed dependent on the IL10 polymorphism and their genotypes. Subjects with a child born before the date of marriage were excluded from analysis regarding fecundity; this was the case for 8 (2%) of the 357 married males and 34 (4%) of the 759 married females.

In female subjects the IL10 –2849 AA genotype was found to be associated with a decreased effective fecundability (longer time period between marriage and first-born child) compared to G allele carriers. Of the 31 female subjects carrying the –2849 AA genotype, 2 (7%) had a calculated conception time of 3 months or less, compared to 116 (28%) of the 410 G allele carriers (OR: 0.2, 95% CI: 0.04-0.7). After adjusting for age at marriage the results remained similar (OR 0.2, 95% CI: 0.04-0.8). At a haplotypic level no significant differences for fecundity could be found. If –2849AA was analysed as homozygous factor compared to the other haplotypes, the results were similar to the results done at single SNP level (OR: 0.2, 95% CI: 0.04-0.9).

Table III. Association between IL10 genotype and effective fecundity.

	Genotype*			OR (95% CI)		
	11	12	22	11 versus rest	22 versus rest	22 versus rest adjusted**
Females						
IL10 G-2849A						
Conception ≤3 months	59 (28)	57 (28)	2 (7)	1.2 (0.8-1.8)	0.2 (0.04-0.7)	0.2 (0.04-0.8)
Conception >3 months	149(72)	145(72)	29 (94)			
IL10 G-1082A						
Conception ≤3 months	28 (23)	61 (28)	30 (33)	0.7 (0.4-1.2)	1.4 (0.8-2.3)	1.4 (0.9-2.4)
Conception >3 months	94 (77)	157(72)	61 (67)			
IL10 C-592A						
Conception ≤3 months	73 (27)	40 (28)	5 (31)	0.9 (0.6-1.5)	1.2 (0.4-3.5)	1.2 (0.4-3.6)
Conception >3 months	196(73)	103(72)	11 (69)			
Males						
IL10 G-2849A						
Conception ≤ 3 months	21 (20)	21 (21)	7 (28)			
Conception > 3months	82 (80)	77 (79)	18 (72)	0.9 (0.4-1.7)	1.5 (0.6-3.8)	1.4 (0.6-3.6)
IL10 G-1082A						
Conception ≤3 months	12 (25)	28 (25)	6 (11)			
Conception >3 months	36 (75)	84 (75)	49 (89)	1.3 (0.6-2.9)	0.4 (0.1-0.9)	0.4 (0.1-0.9)
IL10 C-592A						
Conception ≤3 months	27 (20)	19 (27)	2 (17)			
Conception >3 months	106(80)	52 (73)	10 (83)	0.8 (0.4-1.5)	0.7 (0.1-3.2)	0.7 (0.2-3.4)

Values n (%), OR = Odds Ratio, 95% CI = 95% Confidence Interval.
*The -2849 genotypes are: 11 equals -2849 GG, 12 equals -2849 GA, 22 equals -2849 AA. The -1082 genotypes are: 11 equals -1082 GG, 12 equals -1082 GA, 22 equals -1082 AA. The -592 genotypes are: 11 equals -592 CC, 12 equals -592 CA, 22 equals -592 AA.
** Adjusted by logistic regression for age at marriage.

In male subjects no association between the different IL10 polymorphisms in relation to fecundity could be found. However, in males carrying the -1082AA genotype a decreased

effective fecundability was observed; 6 (11%) of the 55 male –1082 AA genotype carriers had a calculated conception within 3 months of marriage compared to 40 (25%) of the 160 male G allele carriers (OR: 0.4, 95% CI: 0.1-0.9).

DISCUSSION

In the present study we found that female carriers of the IL10 –2849 AA genotype had a significant increase in childlessness and a significant decrease in effective fecundability. Female IL10 –2849 AA carriers were twice as likely to have a marriage that remained childless and were 5 times less likely to have a conception leading to a birth of a child within the first three months of marriage compared to G allele carriers.

Previously the –2849 SNP has been shown to be an important determinant in IL10 responsiveness to endotoxin stimulation with a significantly lower IL10 responsiveness in –2849AA genotype carriers(7). Furthermore an association between reproductive success and a high IL10 responsiveness has been reported previously in addition to a reduced fertility in IL10 –2849 AA genotype carriers(15). The current study confirms and further specifies the influence of the IL10 G–2849A SNP on female fertility. The study was conducted as a continuation on the previous study, comprising a larger cohort with additional information per included subject. It was therefore possible to correct for possible confounders such as age at marriage and age at birth of the first child. Additionally a comparison between males and females was made in relation to fertility, fecundity and the various IL10 polymorphisms.

Female subjects were found to be twice as likely to remain childless when carrying the -2849AA genotype compared to female G allele carriers. The results remained equally significant when correcting for age at marriage. Additionally, it was found that female IL10 -2849AA genotype carriers were 5 times less likely to have a conception within the first 3 months of marriage compared to the G allele carriers, in other words their effective fecundability was significantly lower. The outcome remained equal after correction for age at marriage. These results not only replicate the earlier published results(15;20), but further strengthen the likelihood of a positive link between the IL10 -2849 polymorphism and human reproduction. The relation between non-exon SNPs and gene function is difficult to prove by

biochemical or molecular biological methods because it is not known which stimulus leads to the increased IL10 secretion during pregnancy. Thus methods to prove that a SNP is changing a transcription factor binding site or the rate of transcription of an allele may not be relevant for this specific biological process. Therefore we examined whether IL10 -A2849G was merely a tag of a haplotype or whether it alone was the best predictor of fertility characteristics. Indeed, no relation between the IL10 haplotypes and fertility or fecundity could be found, indicating that the IL10 -A2849G SNP itself is related to affected gene function with regard to fertility. A final conclusion cannot be made as this was generated by a limited number of SNPs. However the low IL10 responsiveness that is particularly found in relation to the IL10 –2849 AA genotype is also compatible with the epidemiological data on low fertility and fecundity associated with the -2849AA genotype. A low IL10 responsiveness may reduce the chance of developing a successful pregnancy.

A low IL10 responsiveness has been reported in relation to recurrent miscarriages. The number of miscarriages or fetal losses could not be analysed with this study design as only births were recorded. The possibility exists therefore, that the effective fecundability (increased interval between marriage and first birth) is reduced due to an increase in the occurrence of miscarriages in -2849 AA carriers. Therefore the exact reason for the decreased fecundity in female IL10–2849 AA genotype carriers remains speculative. No significant association was found when analysing the remaining polymorphisms in association with fertility or fecundity in female subjects.

IL10 has been reported in human semen. Human seminal plasma possesses a generalized immunosuppressive activity(21;22). An important aspect of IL10 in the male genital tract is thought to be the maintaining of the immunological balance and avoid rejection of the spermatozoa(23). Levels of IL10 have been reported lower in semen of infertile men compared to fertile men(24). It has been postulated that a decrease in the presence of IL10 could alter the tolerance to sperm cells in the female genital tract and reduce the favourable condition for fertilisation and implantation. To our knowledge no studies concerning IL10 SNPs and male fertility or fecundity have been published. It would appear likely that a low IL10 responsiveness would decrease male fecundity, with an expectation of finding a relation between male fecundity and the –2849 SNP. The results of the current study however,

revealed no association involving the IL10 –G2849A SNP and the IL10 –C592A SNP in relation to fertility and fecundity in men. An association between the IL10 G-1082A SNP and fecundity in male subjects was seen; men with the IL10 –1082 AA genotype had a decreased effective fecundability compared to G allele carriers. Given the number of comparisons made in this data set and the limited number of subjects, it is most likely a false positive chance finding. If however this finding is a true one, the explanation may lie in the direction of the –1082 SNP interfering with IL10 responsiveness specifically in seminal fluid. No studies have been reported on this topic. Possibly an altered IL10 level in the seminal fluid might interfere with the probability of successful fertilisation, anywhere from the survival of the spermatozoa in the female genital tract to the penetration of the zona pellucidum of the ovum, altering fecundity.

The current study has some limitations. All reproductive information was acquired from registries; therefore all conception times and fecundity rates were calculated. We have no information on pregnancy failures, both miscarriages and stillbirths. The selected cohort was set in a time represented by minimal fertility control and no modern contraceptive methods. We have assumed that starting a family as soon as a marriage was celebrated was desired. The circumstance of the subjects at the time of their marriage is unknown. Any significant illnesses or availability of either partner in the first year after marriage is unknown. Other factors interfering with fecundity as sperm count, regularity of menstrual cycle, frequency of intercourse is unknown.

In conclusion, we found that female IL10 -2849AA genotype carriers were twice as likely to remain childless compared to G allele carriers. Furthermore, female -2849AA genotype carriers were also 5 times less likely to have a conception within the first 3 months of marriage compared to the G allele carriers. These two findings combined clearly confirm that an association between the IL10 –2849 SNP and female fertility exists. Most likely this is due to a decreased IL10 responsiveness found in IL10 –2849 AA genotype carriers, reducing the immunoregulation at the maternal fetal interface decreasing the likelihood of a successful pregnancy.

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4

Miscarriage but not fecundity is associated with progression of joint destruction in rheumatoid arthritis

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ABSTRACT

Objective: To determine whether reproductive history prior to disease onset is associated with severity of joint destruction in rheumatoid arthritis (RA).

Methods: At the department of Rheumatology of the Leiden University Medical Center a special early arthritis clinic (EAC) was established. General practitioners were encouraged to refer patients with joint complaints to this clinic. Subsequently, the diagnosis RA was made by a rheumatologist. Of this cohort 113 female patients with definite RA were included in the current study. A structured questionnaire was taken and the joint damage was measured by sequential X-rays of hands and feet, using the modified Sharp score.

Results: The time of unprotected intercourse until first pregnancy (fecundity) was comparable with data of earlier studies, 16% of the RA patients reported a time to first pregnancy of more than 12 months. Fecundity did not reflect to the extent of joint damage over time. The miscarriage rate was 15% per pregnancy, comparable to population figures (12-15%). A significant increase in joint damage over a 2 year follow-up was found in RA patients who had experienced at least one miscarriage compared to patients who never had a miscarriage in the past (mean modified Sharp score at 2 years 24 (95% CI:15-32) and 16 (95% CI:10-23) respectively, $P<0.05$). At baseline the Sharp scores were similar in both subgroups.

Conclusion: Miscarriage prior to disease onset and not fecundity is associated with the progression of joint damage in RA patients.

INTRODUCTION

The balance between T helper-1 (Th-1) and T helper-2 (Th-2) production regulates various inflammatory responses in humans. Inborn differences in the Th-1/Th-2 balance may be present in Rheumatoid Arthritis (RA) patients with a more predominant Th1 activity(1). This Th1 phenotype is likely to exist form birth onwards and may protect against lethal infectious diseases all through life(2). A more profound Th2 activity is been suggested to be beneficial to the course of RA, as a lower amount of atopic disorders, known to be associated with Th2 predominance, was reported in RA patients(3). Furthermore, a reduced RA disease severity was found in patients whose atopy commenced before their RA development, suggesting an innate Th2 responsiveness(4). A Th2 immune response is likely to be of importance for a successful pregnancy(5). This predominant Th2 response could explain the ameliorating effect on established RA during pregnancy(6). Moreover, Th1 phenotype may result in aberrant characteristics in reproductive history before RA disease onset. This may be expressed in a decreased fertility (ability to become pregnant), fecundity (time to achieve pregnancy from the start of unprotected intercourse) and an increased miscarriage rate.

In 1965 Kay and Bach(7) reported a reduced fertility in pre-menopausal RA patients before and after the onset of RA. However, in a study reported in 1989, subfertility (not pregnant after two years of unprotected intercourse) did not occur more frequent in 117 RA patients compared to controls(8). Fertility in parous women does not seem reduced, as a smaller family size in RA patients has not been observed(9;10). Nulliparity has been reported to be associated to RA, with a consistent odds rate of around 2 for RA in nulliparous women compared to parous women(11). However, whether nulliparity is due to infertility, miscarriages or a choice to remain childless is not clear.

A decreased fecundity (time to achieve pregnancy from the start of unprotected intercourse > 12 months) prior to disease onset was reported in a study in 1993 of 259 RA patients compared to 1258 healthy controls(12). However, this was not confirmed in a study in 1999 where fecundity (> 12 months) in 167 RA patients was comparable to 105 neighbourhood

controls(13). Thus, the impact of RA on fertility and fecundity before disease onset has not been fully elucidated yet.

Miscarriage does not seem to occur more often in women who later develop RA compared to normal population controls(7;8;10;13-16). One American study however, did report a significantly higher number of miscarriages in RA patients but they were not compared to normal population controls but compared to patients with osteoarthritis and other musculosceletal conditions(17).

As many variations may effect human reproduction, as physiological, behavioral, demographic and environmental factors(18), a decreased fertility, fecundity and miscarriage rate in RA patients could also be due to inborn factors linked to RA, even before disease onset. A possible inborn Th1 phenotype may influence both reproductive and RA severity at different ages in life. Severity of RA has not been investigated so far in relation with reproductive success. The aim of the present study was therefore to investigate whether a less favorable reproductive outcome is associated with a more severe RA development. Thus, the reproductive history of women with newly diagnosed RA was studied in relation to the rate of joint destruction.

PATIENTS AND METHODS

Patients. In 1993, a special Early Arthritis Clinic (EAC) was started at the Department of Rheumatology of the Leiden University Medical Center, the only center for rheumatic patients in the rural area of Leiden and environs with 300,000 inhabitants. The general practitioners (GP's) were motivated to refer patients if at least two of the following features were present: joint pain, joint swelling and reduction of joint mobility. All patients referred to the special EAC by the GP's were seen within two weeks. The patients were included in the EAC if 1) arthritis was confirmed by a rheumatologist; 2) the history of symptoms lasted less than two years and 3) the patients had not been visiting a rheumatologist elsewhere for the same problem, to rule out second opinions. Subsequently, the diagnosis "definite RA" was made according to the 1987 ACR criteria(19) but without the requirement of a 6 weeks observation period of arthritis by a rheumatologist(20).

From 1993 to 1999, 644 consecutive patients were included in the EAC with a minimal follow-up of one year, of whom 379 were women. Of these 379 patients, 190 patients were excluded because they had did not have definite RA. Furthermore 8 patients had died (mean age 74 years old), 13 were lost to follow up (mean age 53 years old), 22 refused to participate after informed consent (mean age 67 years old), one patient was excluded because of language difficulties (51 years old) and 32 patients reported to have never had unprotected intercourse with the purpose to achieve a desired pregnancy (mean age 43 years old). This left 113 definite RA patients with a history of unprotected intercourse to be analyzed.

Reproductive history questionnaire. All 113 women were interviewed in respect to their reproductive history. The interview included questions concerning the number of pregnancies, the interval of unprotected intercourse until first pregnancy, number of pregnancy losses and the age during pregnancies. Time to pregnancy (fecundity) was defined as the self-reported time between child wish and unprotected intercourse and the occurrence of pregnancy. Fecundity was calculated for pregnancies that ended in the birth of a child as well as for pregnancies ending in miscarriage. Miscarriage was defined as the loss of a pregnancy prior to 20 weeks. The same person (DR), who was blinded for the diagnosis, interviewed all patients.

Assessment of outcome. The primary outcome was the radiographic joint damage, measured by the modified Sharp score(21). Radiographs of the hands and feet were taken at the time of diagnosis, at 6 months, at one year and at two year follow up. The radiographs were scored in random order by an experienced rheumatologist blind to the clinical data and not aware of the study questions. The intraclass correlation coefficient for the radiograph reading of the assessor was 0.95.

Secondary outcomes were a modified disease activity score(22) and the C-reactive protein (CRP) level at inclusion and during follow up. The disease activity score (DAS) was calculated as 0.53 * (Ritchie score)^{½} + 0.065 * (number of swollen joints) + 033 * ln erythrocyte sedimentation rate + 0.224. All joints were assessed as in the Ritchie Articular Index, except for the acromioclavicular, subtalar and midtarsal joints. For the swollen joint index the metacarpophalangeal, proximal interphalangeal and metatarsophalangeal joints were scored as one unit.

Statistical Analysis. The Statistical Package for Social Science (SPSS) was used for analysis of the results. The subgroups were tested using the Pearson's Chi-square test and Mann-Whitney U test, accordingly. Differences between the Sharp scores of the subgroups were tested with Mann-Whitney U test. All tests were 2-tailed and a P value less than 0.05 was considered significant.

RESULTS

Demographic and reproductive characteristics. One hundred and thirteen female patients with RA were included in this study. The general characteristics of all RA patients are shown in Table 1.

Table 1 Demographic and reproductive characteristics of the patients with rheumatoid arthritis at baseline.

	RA patients
Number of subjects	113
Ever pregnant (%)	110 (97)
Mean age at interview (SD)	59 (15)
Mean age at 1st visit (SD)	55 (15)
Mean duration of complaints at 1st visit in days (SD)	200 (160)
DMARD use (%)	99 (88)
Interval until start 1st DMARD in days (SD)	92 (144)
Rheumatoid factor positivity (%)	61 (54)
Pregnant before RA onset (%)	106 (94)

SD= standard deviation

DMARDs: disease modifying antirheumatic drugs

Fertility. Three patients (3%) had not achieved a desired pregnancy. They were 29, 30 and 37 years old at interview and had all been trying to conceive for more than a year. They had not had any fertility treatment yet.

Fecundity in relation to joint damage in RA. Of the 110 patients who had been pregnant at least once, 70 (62%) had reported that the time to their first pregnancy had been 3 months or less, 20 (18%) patients reported it had been 4 to 12 months and 18 (16%) patients reported it had been more than 12 months. Two patients could not recall the time it took to achieve their first pregnancy. When divided in groups according to their history of fecundity, the patient characteristics were similar in all subgroups (data not shown). Measuring the joint damage over time using the modified Sharp score (0, 6, 12 and 24 months), there was no difference in the development of joint destruction in these three fecundity groups (Table 2). When the patient group with a fecundity ≤ 12 months was compared to the patient group with a fecundity >12 months, the mean Sharp score was comparable (at baseline: mean Sharp score 4 (95% confidence interval [CI]:2-7) and 8 (95% CI:-5-21), respectively and at two years: mean Sharp score 17 (95% CI:12-23) and 25 (95% CI:7-43), respectively).

Table 2 Mean Sharp scores for 110 patients with rheumatoid arthritis according to fecundity

	Sharp at baseline	Sharp at 6 months	Sharp at 12 months	Sharp at 24 months
Fecundity				
< 3 months (n=70)	5.0 (1.3)	9.7 (2.2)	15.7 (2.7)	19.3 (3.3)
4 – 12 months (n=20)	2.2 (0.9)	3.8 (1.3)	5.4 (1.3)	11.9 (3.2)
> 12 months (n=18)	8.3 (6.1)	10.1 (5.3)	21.3 (10.2)	25.0 (8.2)

Miscarriage in relation to disease severity in RA: Joint damage. The miscarriage rate per pregnancy was 15% for the 110 RA patients with at least one pregnancy in the past. The patient characteristics were comparable when these patients were divided according to their miscarriage history (Table 3). The patient group with at least one miscarriage understandably

experienced significantly more pregnancies to achieve a similar mean amount of children (Table 3).

Table 3 Demographic and reproductive characteristics of patients with rheumatoid arthritis with at least one pregnancy (n = 110) at baseline, divided by history of miscarriages.

	0 Miscarriages (n = 74)	≥ 1 Miscarriages (n = 36)
Mean age at interview (SD)	59 (14)	62 (15)
Mean age at 1st visit (SD)	55 (14)	58 (15)
Mean duration of complaints at 1st visit in days (SD)	186 (149)	236 (181)
DMARD use (%)	63 (85)	33 (92)
Interval until start 1st DMARD in days (SD)	84 (107)	109,3 (203)
Rheumatoid factor positivity (%)	38 (55)	23 (66)
Pregnant before RA onset (%)	72 (97)	34 (94)
Mean age at 1st pregnancy (SD)	26 (5)	27 (5)
Mean number of pregnancies (SD)	2,3 (1.1)	4,1* (1.9)
Mean number of children (SD)	2,3 (1.1)	2,8 (1.9)
Marital status, currently married (%)	59 (80)	26 (72)
Ever smoked (%)	20 (27)	6 (17)
Education: college or university (%)	18 (24)	9 (25)

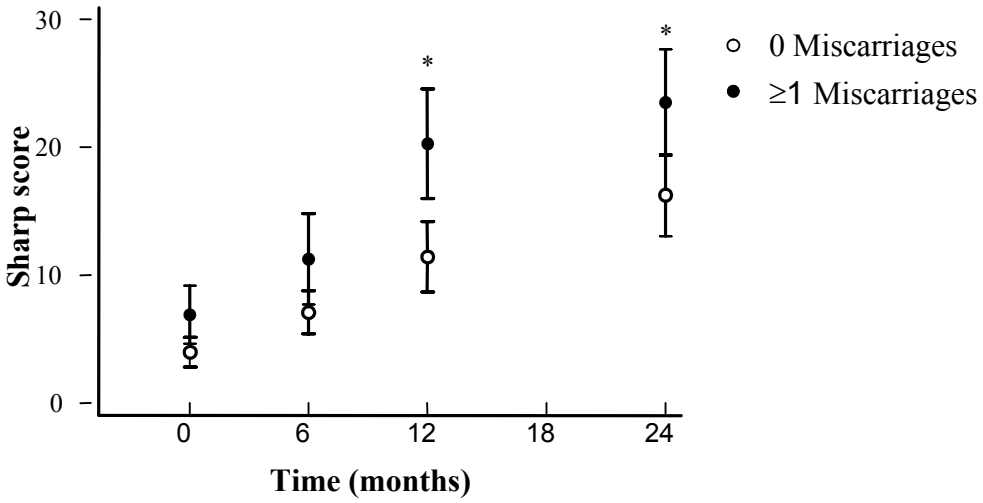
SD= standard deviation, DMARDs = disease modifying antirheumatic drugs

* P<0,05, compared to the group without miscarriages.

The modified Sharp score over a two-year period was analyzed (0, 6, 12 and 24 months) for each of the subgroups. A significant increase in joint destruction was found in women who had experienced at least one miscarriage in the past compared to women who did not have any miscarriages in the past (Figure 1). At inclusion the mean Sharp score was 4 (95% CI: 1-

7) for the group without miscarriages and 7 (95% CI: 2-11) for the group with at least 1 miscarriage. The group without miscarriages progressed to a mean Sharp score of 16 (95% CI: 10-23) after two years follow-up, while the group with at least one miscarriage progressed to a mean Sharp score of 24 (95% CI: 15-32; $P<0.05$ compared with the group without miscarriages). The outcome was similar when patients were excluded who had their first pregnancy after the onset of the disease (n=4). A multivariate analysis was preformed for prognostic factors as age at onset, duration of complaints before referral, rheumatoid factor and shared epitope, this did not alter the outcome significantly.

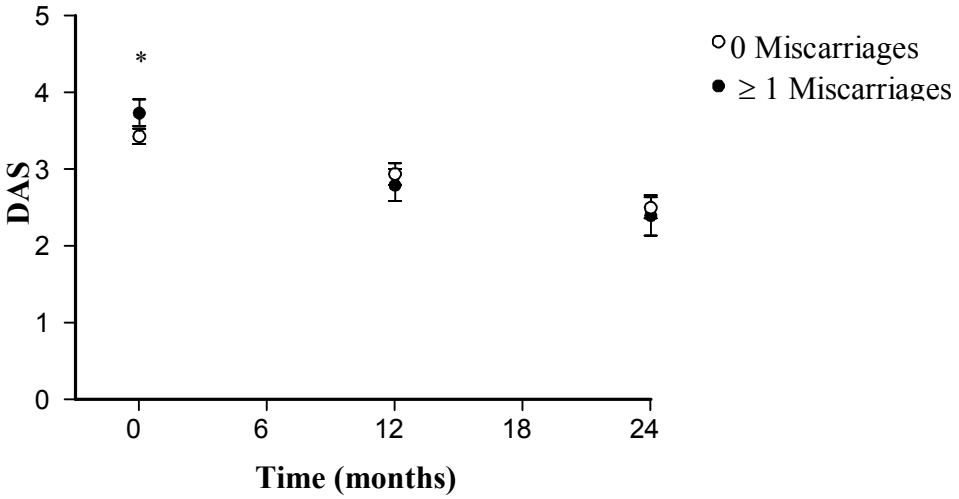
Figure 1 Mean Sharp scores over time as a measure of joint damage in patients with rheumatoid arthritis according to their history of miscarriages (*p<0.05 for the two subgroups). Error bars = SEM.



Miscarriage in relation to disease severity in RA: Activity outcome of disease. The mean DAS at baseline was 3.4 (95% CI: 3.2-3.6) for the group without miscarriages and 3.7 (95% CI: 3.4-4.1; $P=0.05$) for the group with at least one miscarriage (Figure 2). The DAS improved in both groups and were comparable for the two subgroups for the duration of the follow-up period of 2 years (2.5, 95% CI: 2.2-2.8 and 2.4, 95% CI: 1.9-2.9, respectively at 2 years). At baseline the mean CRP level (Figure 3) was significantly lower in the group without miscarriages (24, 95% CI: 17-30) compared to the group with at least one miscarriage

(41, 95% CI: 27-55, *P*<0.05). During the two years follow up the CRP levels were similar in both groups (15, 95% CI: 8-22 and 18, 95% CI: 5-31, respectively).

Figure 2. Mean (standard error) disease activity scores during follow up in RA patients according to their history of miscarriages (* P< 0.05 for the two subgroups).



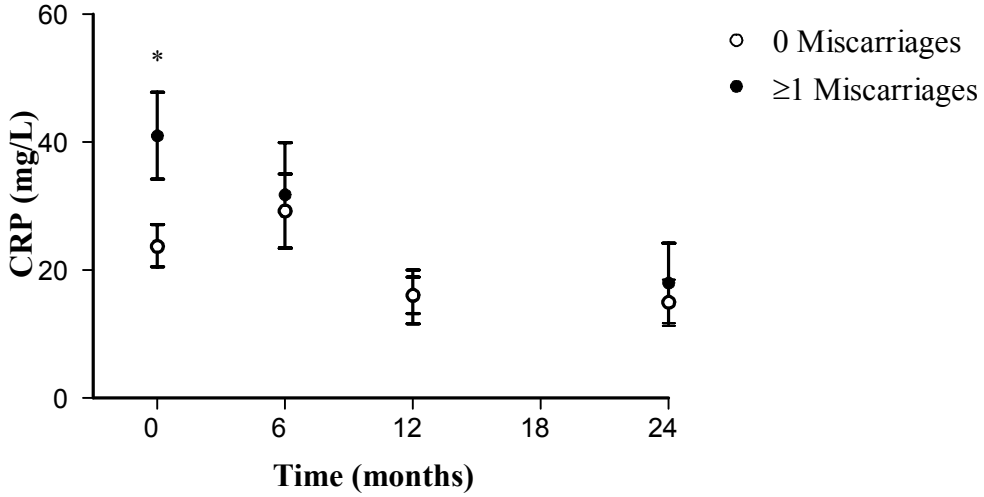
DISCUSSION

In this study, we observed that a high rate of joint damage in RA patients is associated with a history of miscarriage but not with history of a prolonged fecundity. To our knowledge this is the first study in which the severity in disease course of RA patients, as measured by rate of joint destruction, is related to reproductive history.

The miscarriage rate per pregnancy in our study was 15%, which is consistent with the miscarriage rate of 12-15% in the normal population(23). In the literature the miscarriage rate has been reported to be comparable between RA patients and healthy population control groups(7;8;10;13-16). However, we did find a relationship between miscarriage and severity of RA. The history of at least one miscarriage increased the rate of joint damage in RA patients by 2-fold over a 2 year follow-up period. Two years is a relatively short time to assess outcome and a longer follow up would have been preferable, although radiological

damage is known to occur early in RA. The association between miscarriage and severity of joint damage in RA could not be explained by a significant difference in the duration of complaints before inclusion, or by a difference in treatment strategy between the two groups. At baseline the group with at least one miscarriage had a significantly higher CRP level and a significantly higher DAS relative to the group without a miscarriage, indicating that this subgroup had more severe symptoms at first visit. At follow-up however, both CRP and DAS decreased to similar levels in both subgroups, indicating that the RA symptoms were treated sufficiently in both subgroups. The radiographic joint damage using the modified Sharp score was the only measure that was similar at baseline and progressed significantly in the group with at least one miscarriage at 1 and 2 years follow-up, compared to the group without miscarriages. A history of miscarriage may represent a group with a more severe disease activity, which will lead to a higher progression in joint damage, possibly reflecting the Th1 phenotype in this subgroup.

Figure 3 Mean C reactive protein concentrations during follow up in the patients with rheumatoid arthritis according to their history of miscarriages (*p<0.05 for the two subgroups). Error bars = SEM.



This cytokine mechanism could explain the difference in progressive joint damage in the two subgroups. An inborn predominant Th-1 response in the miscarriage subgroup may be harmful to both the disease process in the joint and to the physiological immunological

changes during pregnancy, even before clinical disease manifestation. A predominant Th2 response is necessary for normal pregnancy, this predominance is less clear in pregnancies undergoing spontaneous abortion(24). The probability of normal fecundity increased more than tenfold when the innate cytokine profile was characterized by a Th2 responsiveness, compared to the profile of women with recurrent abortion, whose cytokine profile was characterized by low Th2 and high Th1 responsiveness(25). A more pronounced inborn Th-1 profile may characterize women who experience miscarriage and if these women develop RA, it is conceivable that their RA is characterized by more extensive joint destruction.

A decreased fertility rate is difficult to assess, as the ability to have a child is dependent on numerous factors both related to male and female factors. In our study 16% of the RA patients reported a time to pregnancy of more than 12 months. Fecundity in the normal population is reported to be 58-65% for the first 3 months, 85-90% for the first 12 months and the remaining 10-15% have a time to pregnancy of more than a year(26;27). Decreased fecundity (prolonged interval until desired pregnancy) in RA patients seems plausible(12;28). The 16% reported in our study is comparable, however, a population control group was not available. A probable decreased fecundity rate in RA may represent the effect of an inborn characteristic in these RA patients. However, a relation to disease severity and joint damage could not be revealed in this study, possibly due to the small numbers in this subgroup.

In the current retrospective study, reproductive data were collected through interview. Time to pregnancy measured in months is known to be a sensitive measure of the biological fertility of a couple(26). Recall bias is possible, however, validation studies of fecundity and miscarriages have shown a good match between long-term recall (>15 years) of personal reproductive history collected either through personal interview, telephone interview or written questionnaire compared to medical data(29;30). Even though our group consisted of a different patient group at a different time period, we presumed that these validation studies are applicable on our study.

In summary, the current study indicates that miscarriages prior to disease onset in RA patients is comparable to what is reported in the normal population, but after developing RA, a history of miscarriage may lead to a higher rate of joint destruction. Fecundity seems to be decreased in RA patients prior to disease onset, however this study failed to reveal a relation to joint

damage, possibly due to small numbers. The results could indicate that the phenotype of joint destruction is associated with the phenotype of reported miscarriages, suggesting common genetic risk factors for each of these two traits, possibly through the innate Th-1/Th-2 phenotype.

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5

Factor V Leiden Mutation in Relation to Fecundity and Miscarriage in Women with Venous Thrombosis

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Hum Reprod. 2005 Mar; 20(3): 802-6

ABSTRACT

BACKGROUND: Factor V Leiden mutation (Arg506Gln) increases the likelihood of venous thrombosis. Factor V Leiden mutation may also have a positive effect through facilitation of embryo implantation. This may manifest itself as a reduced time to pregnancy (increased fecundity) and fewer miscarriages in the first trimester.

METHODS: From March 1999 onwards, consecutive patients with a first venous thrombosis (VT) were recruited. The first 115 female VT patients with factor V Leiden and 230 age-matched female VT patients without factor V Leiden were included. All patients, unaware of their genotype, received a structured questionnaire.

RESULTS: 297 (86%) Women returned the questionnaire, 220 had been pregnant at least once. Time to first pregnancy was unaffected by carrier status: 58% factor V Leiden carriers reported a pregnancy within 3 months compared to 54% non-carriers. The miscarriage proportion was 14%, similar in both groups. First trimester miscarriage was less frequent among carriers (46%) than among non-carriers (95%) (RR 0.5, 95% CI: 0.3–0.9).

CONCLUSIONS: Factor V Leiden mutation may support embryo implantation, as factor V Leiden carriers had fewer miscarriages in the first trimester with a similar overall miscarriage rate. Miscarriage of embryos with poor viability may be postponed until the second trimester in factor V Leiden carriers. Fecundity was not influenced by factor V Leiden status.

INTRODUCTION

Factor V Leiden mutation (Arg506Gln) is present in 4 to 10% of people of Caucasian origin (Bertina *et al.,* 1994; Rees, 1996). The factor V Leiden mutation induces a hypercoagulable state which increases the risk of venous thrombosis seven-fold among heterozygous carriers and about eighty-fold among homozygous carriers compared to non-carriers (Rosendaal *et al.,* 1995). It has been suggested that factor V Leiden mutation may be associated with negative outcomes of reproduction such as (recurrent) abortion, pre-eclampsia, prematurity and small-for-gestational-age neonates. However, the available data are conflicting (Pauer *et al.,* 2003; Hundsdoerfer *et al.,* 2003; Morrison *et al.,* 2002; Rai *et al.,* 2001). Because of the high prevalence of this mutation in certain populations positive effects associated with factor V Leiden have been postulated, possibly through human reproduction. Women who carry the factor V Leiden mutation lose less blood in menstruation, have higher haemoglobin levels, and possibly a lower incidence of life threatening post-partum haemorrhage which could be an evolutionary advantage (Lindqvist *et al*., 2001). Furthermore, a higher than expected prevalence of factor V Leiden mutation carriers was found in healthy pregnant women (9.2%) (De Groot *et al.,* 1999) compared to general population figures (3%) (Rosendaal *et al.,* 1995). A similar finding was reported in a recurrent miscarriage study where the prevalence of factor V Leiden was higher in women without a history of recurrent miscarriages (14%) compared to those with recurrent miscarriages (1.7%) (Dilley *et al.,* 2002). Facilitation of embryo implantation was suggested as a possible positive pathway for factor V Leiden (Majerus, 1994). In agreement with this hypothesis an improved implantation rate in factor V Leiden carriers compared to non-carriers was reported in intra-cytoplasmatic sperm injection (ICSI) pregnancies. If either the mother or the fetus carried the factor V Leiden mutation the proportion of live births was 90% (9/10) for the first embryo transfer compared to 49% (45/92) in factor V Leiden negative pairs (Göpel *et al.,* 2001). A reduced time to pregnancy (increased fecundity) in spontaneous pregnancies of factor V Leiden carriers would be an indication of a protective effect of factor V Leiden.

To study the effect of factor V Leiden mutation on embryo implantation and human reproduction, we investigated the association of factor V Leiden mutation on fecundity and miscarriages and the trimester in which the miscarriages occurred in 297 women. To be able

to construct a large cohort of women with factor V Leiden, we used information from a large study on venous thrombosis. We also investigated the effect of factor V Leiden carriership on term birth rate and birth weight.

MATERIALS AND METHODS

The women described in this study were enrolled in the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis study (MEGA study). The primary aim of the MEGA study is to assess interaction between environmental and genetic risk factors for venous thrombosis. From March 1999 onwards, all consecutive patients who suffered a first deep-vein thrombosis or pulmonary embolism between the age of 18 and 70 were recruited from six anticoagulation clinics in The Netherlands. The anticoagulation clinics monitor the anticoagulant therapy of all patients in a well-defined geographical area, which allowed us to identify consecutive and unselected patients with venous thrombosis. All participants filled in a questionnaire on risk factors for venous thrombosis. A blood sample was drawn three months after discontinuation of anticoagulation. Subjects who were unable or declined to give a blood sample, were given the opportunity to give DNA by use of a buccal swab. All participants completed an informed consent form. The Leiden University Medical Center medical ethical committee approved of the study.

For the present analysis, the first 115 female thrombosis patients identified with factor V Leiden mutation were matched on age to 230 female thrombosis patients (controls). Besides the age matching and absence of factor V Leiden, controls were randomly selected from the study participants. All 345 patients, who were unaware of their genotype, were asked to fill in an additional structured questionnaire concerning their reproductive history. Questions consisted of age at first pregnancy attempt, the period of unprotected intercourse until the desired pregnancy occurred, number of pregnancies, and the duration of each pregnancy. When there had been no pregnancies, we enquired whether this was despite efforts to become pregnant (infertility). If there was no response to the initial questionnaire, a written reminder accompanied with an identical questionnaire was sent after three weeks. To increase response,

patients were contacted by telephone if they did not return the questionnaire after five additional weeks.

Time to pregnancy was defined as self-reported time between unprotected intercourse and the occurrence of pregnancy. Miscarriage ratio was calculated as the total number of miscarriages per number of pregnancies. First trimester miscarriage was defined as embryonic or fetal loss before the completion of 12 weeks gestation, second trimester miscarriage was defined as fetal loss from 13 to 24 weeks gestation, stillbirth was defined as a loss after 24 weeks gestation. A number of factors known to influence miscarriage or fecundity were included in the questionnaire, such as age at first pregnancy attempt, age at first birth, level of education (primary school, secondary school, college or university), smoking habits, and alcohol use. The body-mass index (BMI) was calculated (weight/height^2) from the weight (in kg) and height (in m) obtained at the time of thrombosis.

DNA was isolated from whole blood or buccal swabs. For the latter, three large cotton swabs in a total of 6 ml SDS-proteinase K solution (100 mM NaCl, 10 mM EDTA, 10 mM Tris-HCl, pH = 8.0, 0.5% SDS, 0.1 mg/ml proteinase K) were obtained from each patient. Upon arrival, the proteinase K concentration was raised to 0.2 mg/ml and the sample was incubated for 2 hrs at 65°C. Subsequently, the suspension was recovered by centrifugation. Potassium acetate was added to a final concentration of 1.6M. After 15 min incubation on ice, proteins were removed using chloroform/ isoamylalcohol (24:1) treatment. The DNA in the water-phase was subsequently ethanol precipitated. After centrifugation, the pellet was resuspended in 200 µl 10 mM Tris-HCl, 10 mM EDTA pH=8.0 and frozen at -20ºC until further analysis. Assessment of the factor V Leiden mutation in DNA retrieved from the buccal swabs was performed identically to the method for DNA from whole blood, and determined by polymerase chain reaction (PCR) and *Mnl I* restriction digestion as described elsewhere (Bertina *et al.,* 1994).

Data are presented as simple counts and percentages. Relative risks were computed as the ratio of these counts, and 95% confidence intervals were based on a binomial distribution.

RESULTS

Two hundred and ninety-seven (86%) women returned a completed questionnaire, 89 % of the factor V Leiden carriers and 84% of the non-carriers. Of the 48 non-responders, 4 women were deceased, 6 were lost to follow-up and for 21 women, the reason for not returning the questionnaire was unknown. Seventeen questionnaires were sent back blank, for reasons of lack of motivation (4 women), 3 women were too ill, for 2 women it brought back too many painful memories (both non-carriers), and in 8 cases no reasons were given.

Table I. Patient characteristics and reproductive outcome of the 220 women who had been pregnant, according to factor V Leiden carrier status

	Factor V Leiden carrier [a] n = 80	Factor V Leiden non carrier n = 140
Age at thrombosis, years	45.1 (20-68)	44.4(20-70)
Age at questionnaire, years	47.6 (23-71)	46.8 (23-72)
Menstrual cycle, regular (%)	66 (84)	118 (84)
Education, college/ university (%)	32 (45)	52 (43)
Smoked, ever (%)	50 (63)	77 (55)
BMI, kg/m2	27 (18-58)	27 (17-57)
Age at 1st pregnancy attempt, years	24.1 (15-36)	24.1 (15-38)
Age at birth first child, years	25.2 (16-36)	25.3 (15-39)
Number of pregnancies	2.2	2.7
Number of liveborn children	1.7	1.9
Total number of miscarriages (%)	25 (14)	50 (14)
Stillbirths (%)	2 (1)	9 (2)
Ectopic pregnancies (%)	0 (-)	4 (1)
Planned Abortion (%)	10 (6)	17 (4)
Other, (twins eg.) (%)	4 (2)	9 (2)

Values are mean (range) or *n* (%). [a] One homozygous factor V Leiden mutation carrier.

Of the 102 factor V Leiden carriers who returned the completed questionnaire, 80 (78%) had been pregnant at least once compared to 140 (72%) of the 195 non-carriers (RR 1.1, 95% CI 0.95-1.2). Patient characteristics of these 220 women are listed in Table I. The reasons for not having had a pregnancy were similar; infertility was reported by 5% (1/22) of factor V Leiden carriers and 11% (6/55) of non-carriers (RR 0.4, 95% CI 0.1-3.3).

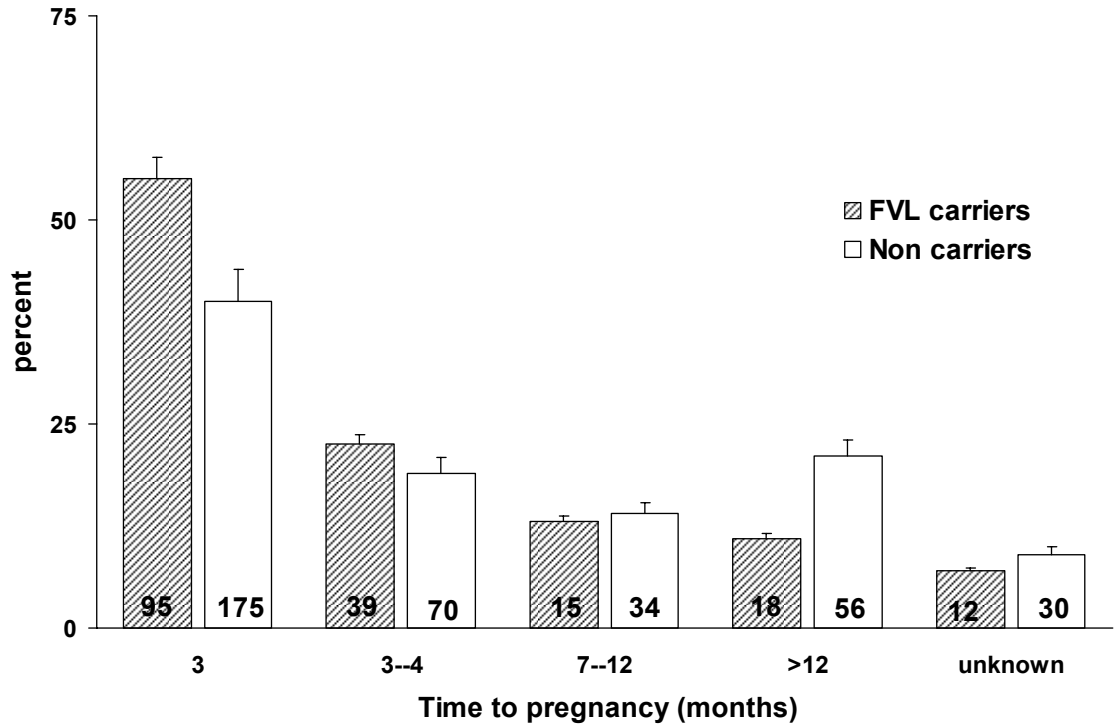

Figure 1. Fecundity (time to pregnancy) for all pregnancies in 220 venous thrombosis patients according to factor V Leiden mutation (179 pregnancies in factor V Leiden carriers and 365 pregnancies in non-carriers, number of pregnancies stated in the bars).

Time to first pregnancy was similar for factor V Leiden carriers and non-carriers. In both groups over 90% of those who had been pregnant could recall the time to their first pregnancy. Forty-two (58%) factor V Leiden carriers achieved their first pregnancy within 3 months compared to 70 (54%) of the non-carriers (RR 1.1, 95% CI 0.8-1.4). Nine (13%) factor V Leiden carriers reported a time to first pregnancy of more than 12 months, which had occurred in 21 (16%) of the non-carriers (RR 0.8, 95% CI 0.4-1.6). When all consecutive pregnancies per woman were combined, similar results were found (Figure 1).

The miscarriage proportion for all pregnancies was similar in both groups, 14% (25/179) for factor V Leiden carriers and 14% (50/365) for non-carriers (RR 1.0, 95% CI 0.6-1.6). Considering only the first pregnancy, 16% (13/80) of the factor V Leiden carriers had experienced a miscarriage compared to 15% (21/140) of the non-carriers (RR 1.1, 95% CI 0.6-2.0). However, the trimester in which the miscarriages occurred was different according to factor V Leiden status; factor V Leiden carriers who experienced a miscarriage during their first pregnancy had fewer miscarriages in the first trimester compared to non-carriers, respectively 46% and 95% (RR 0.5, 95% CI: 0.3-0.9). Subsequently, factor V Leiden carriers had more miscarriages in the second trimester (RR 10.8, 95% CI 1.5-77.7) (Table IIA). When all consecutive pregnancies per woman were combined the difference persisted (Table IIB). Stillbirth (fetal loss in the third trimester) was rare, and was equal in both groups. For the 80 first pregnancies of factor V Leiden carriers no stillbirths occurred compared to four (3%) stillbirths in 140 first pregnancies in non-carriers. For all pregnancies per woman combined, two (1%) stillbirths occurred in 179 pregnancies in factor V Leiden carriers compared to nine (2%) in 365 pregnancies in non-carriers (RR 0.4, 95% CI 0.1-2.1).

Table II. Miscarriages, per trimester, out of 220 women who had been pregnant, according to factor V Leiden (FVL) mutation

	First trimester (≤12 weeks)	Second trimester (13-24 weeks)	Total
First pregnancy only (80 pregnancies in FVL carriers and 140 in non-carriers)			
FVL+ (%)	6 (46)	7 (54)	13
FVL- (%)	19 (95)	1 (5)	20 [a]
Relative risk, (95% CI)	0.5 (0.3-0.9)	10.8 (1.5-77.7)	
All pregnancies combined (179 in FVL carriers and 365 in non-carriers			
FVL+ (%)	16 (64)	9 (36)	25
FVL- (%)	43 (90)	5 (10)	48 [b]
Relative risk (95% CI)	0.7 (0.5-1.0)	3.5 (1.3-9.2)	

Values are *n* (%). [a] Of one non-carrier, the trimester in which the miscarriage took place was unknown. [b] Of two non-carriers, the trimester in which the miscarriage took place was unknown. CI = confidence interval.

The proportion of live births was similar in both groups; 74% (59/80) among factor V Leiden carriers compared to 76% (106/140) among non-carriers (RR 1.0, 95% CI 0.8-1.1). Term birth (37-42 weeks gestation) in first pregnancies was comparable, 64% (38/59) and 69% (73/106), respectively (RR 0.9, 95% CI 0.7-1.2). Mean birth weight for the children born at term was similar for factor V Leiden carriers (3644 gram, range 2500-4000) and non-carriers (3481gram, range 1200-3975).

DISCUSSION

In this study of 297 women with venous thrombosis, we found no association between factor V Leiden mutation and fecundity or the frequency of miscarriage. However, when miscarriages had occurred, they took place less often in the first trimester in factor V Leiden mutation carriers than in non-carriers.

Miscarriages occurred as often in factor V Leiden carriers (14%) and non-carriers (14%), in percentages that are similar to general population figures (10 to 15%) (Zinaman *et al*., 1996). Published data on factor V Leiden in relation to miscarriages are conflicting. Recent meta-analyses have shown a relation between factor V Leiden and recurrent fetal loss, occurring both in early and in late in gestation (respectively OR 2.01, 95% CI 1.13-3.58 and OR 7.83, 2.83-21.67) (Rey *et al*., 2003; Kovalevsky *et al*., 2004). For non-recurrent early loss (< 19 weeks gestation) no clear association was found with factor V Leiden (OR 1.40, 0.66-2.97); and for non-recurrent isolated second/third trimester loss (stillbirth > 19 weeks) a positive association was found (OR 3.26, 1.82-5.83) (Rey *et al*., 2003; Dudding *et al*., 2004).

The focus in literature has mainly been on factor V Leiden in relation to second and third trimester loss, as thrombosis of the placental vessels is assumed to be an important factor in fetal loss. In our study factor V Leiden carriers experienced more fetal loss in the second trimester, as would be expected by the placental vessel thrombosis theory. A clear increase in third trimester loss would be expected too, with an even higher rate of fetal loss in third than in second trimester for factor V Leiden carriers. However, third trimester loss (stillbirth) was equally distributed over carriers and non-carriers, in a comparable small rate, which contradicts this theory. Moreover, in the present study, mean birth weight was not influenced

by factor V Leiden status, which is in line with earlier published data (Lindqvist *et al.,* 1999). If we consider hypercoagulability to lead to obstruction of placental vessels as a major pathological factor among pregnant factor V Leiden carriers, one would not expect an effect on first trimester miscarriages, since the placental circulation in the first trimester has not yet been fully established (Hustin and Schaaps, 1987; Burton *et al.,* 1999). Therefore, impaired placental perfusion might not be critical for the embryonic development and implantation during (very) early gestation. We can not differentiate between the types of early pregnancy loss in this study (eg presence or absence of fetal heart prior to the loss), as all the data were provided to us by the patients through interview. Furthermore, most would have not had an early first trimester scan, as they are not routinely done in The Netherlands.

Our results indicate a clear reduction in first trimester loss in factor V Leiden carriers, which is subsequently compensated by an increased loss in the second trimester. Similar findings were reported in a recent study where a decreased risk of recurrent pregnancy losses at less than 10 weeks gestation was found in factor V Leiden carriers (OR 0.23, 95% CI 0.07-0.77) with a subsequent increase in losses after 10 weeks (Roqué *et al*., 2004). This suggests a protective effect on the embryo during the first trimester in factor V Leiden carriers, including less viable embryos that will eventually abort in the second trimester. This may explain the similar overall frequency of miscarriages in factor V Leiden carriers and non-carriers. A lower frequency of miscarriages in the first trimester may thus reflect a successful implantation. Approximately 50-70% of all miscarriages are attributable to detectable chromosomal abnormalities, furthermore 15-20% is thought to be due to morphological defect(s) in the embryo. A recent study confirmed this with a transcervical embryoscopy at the time of the curette and cytogenetic analysis of the products of conception (Philipp *et al*, 2003). We do not have any cytogenetic information in our study, as karyotyping of the products of conception is not a routine consideration in The Netherlands. The possible protective effect of factor V Leiden early in pregnancy will require further study with among other things, cytogenetic testing of the miscarriage products and possibly transcervical embryoscopy prior to evacuation.

Our study did not show a clearly increased fecundity in factor V Leiden carriers. Many factors affect fecundity, including physiological, behavioural and environmental factors. Known

factors, such as age at pregnancy attempt, regularity of the menstrual cycle, smoking habits and educational level were similar in factor V Leiden carriers and non-carriers. As fecundity is a manifestation of both conception and implantation it is important to further distinguish these. For conception, factors such as sperm quality, coital frequency and timing are of great importance. However, this study was not designed to examine these factors and it remains unclear whether factor V Leiden has any effect on these factors. Aspects influencing implantation have not been fully elucidated yet, however an improved implantation could be due to an increase in the hypercoagulable state, related to the factor V Leiden mutation. This was suggested by a study that omitted conception by reporting only on ICSI pregnancies, reflecting implantation success (Göpel *et al.,* 2001). A higher incidence of implantation success was found if either the mother or the fetus was a factor V Leiden carrier. It is possible that the beneficial effect of factor V Leiden on implantation alone has a less clear affect on fecundity compared to various factors concerning conception and therefore, in our study, fails to show an overall difference in fecundity.

We investigated the reproductive histories of women who had suffered venous thrombosis. The choice of this design was opportunistic, for it offered the opportunity to study a large cohort of factor V Leiden carriers. We have considered whether this choice, rather than the ideal study of factor V Leiden carriers without thrombosis, could have distorted our results. Firstly, the period about which questions were asked preceded the thrombotic event, in most cases by many years. Hence, the thrombotic event itself did not influence our results. As the patients developed thrombosis, they will have more risk factors for thrombosis than other women. It is known that this is not only true for women without factor V Leiden but also for women with factor V Leiden (the majority of people with factor V Leiden never develop thrombosis, and there must be causes why some do). For this reason, we chose thrombosis cases without factor V Leiden as controls rather than healthy women without factor V Leiden, and therefore differences between the group can be attributed to factor V Leiden. One could argue that the groups differed more: women with thrombosis without factor V Leiden probably had more additional risk factors than those with factor V Leiden, for instance another, possibly still unknown, gene defect. This could, if that other prothrombotic factor also affected implantation and fetal loss, explain the absence of a difference between the groups in the frequency of miscarriage. However, we did find a difference. As the

reproductive data in the present study were collected by interview, recall bias is possible, but seems unlikely. Validation studies of fecundity and miscarriages have shown a good match between long-term recall of personal reproductive history through interview and medical data (Joffe *et al*., 1993). Moreover, the women were unaware of their factor V Leiden status at the time of the questionnaire.

In conclusion, factor V Leiden mutation may support embryo implantation, as factor V Leiden carriers reported significantly fewer miscarriages in the first trimester. This was not reflected in an increased fecundity. The overall miscarriage proportion was not influenced by factor V Leiden status. These results suggest that factor V Leiden offers a protective effect on early pregnancy and that the miscarriage of embryos with poor viability in factor V Leiden carriers is postponed until the second trimester.

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6

Gender-specific association of the factor V Leiden mutation with fertility and fecundity in a historic cohort. The Leiden 85-Plus Study

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ABSTRACT

BACKGROUND: Factor V Leiden (FVL, Arg506Gln) mutation may facilitate embryo implantation and increase fertility and fecundity. This was studied in subjects who were of childbearing age in a time with minimal fertility control without modern contraceptive methods.

METHODS: From 1986 to 1999, 1502 inhabitants of Leiden, The Netherlands, reaching the age of 85 years were enrolled in the Leiden 85-Plus Study. Of 1176 subjects the FVL status was analysed, in 365 male and 811 female subjects.

RESULTS: The FVL carrier rate was 4.3%. Fertility was not affected by FVL status. In male subjects, fecundity (interval between marriage and birth of first child) was significantly increased in FVL carriers; 67% of male FVL carriers had a child within 371 days of marriage (therefore conceived within 3 months of marriage), compared with 19% of male non-carriers [relative risk (RR), 3.5; 95% confidence interval (CI), 2.1–5.7; $P < 0.001$]. Within 6 months of marriage, 75% of male FVL carriers had conceived a child compared with 34% male non-carriers (RR, 2.2; 95% CI, 1.5–3.2; $P = 0.01$). In female subjects, fecundity was not influenced by FVL status.

CONCLUSION: Fecundity is increased in male FVL carriers; in female subjects, no such association was observed.

Factor V Leiden (FVL, Arg506Gln) mutation is present in 4–10% of people of Caucasian origin (Bertina *et al*., 1994; Rees, 1996). The FVL mutation induces a hypercoagulable state which increases the risk of venous thrombosis three- to sevenfold among heterozygous carriers and about eightfold among homozygous carriers compared to non-carriers (Rosendaal *et al*., 1995).

The persistence and high prevalence of the FVL mutation in the general population suggests that it may carry an evolutionary advantage. As early as 1957, George Williams proposed the 'antagonistic pleiotropy' theory (Williams, 1957). Briefly, this theory states that ageing is due to the decline of the force of natural selection late in life and that the fixation of alleles with positive effects upon fitness early in life also have deleterious effects late in life. This 'antagonistic pleiotropy' theory may apply to FVL since a positive effect on implantation has been suggested (Majerus, 1994). This positive effect was subsequently verified in a study where an improved implantation rate in ICSI pregnancies was reported if either the mother or the child carried the FVL mutation (Göpel *et al*., 2001).

Some evolutionary benefit of FVL mutation in females may lie in the fact that women who carry the FVL mutation lose less blood in menstruation, have higher haemoglobin levels and possibly have a lower incidence of life-threatening post-partum haemorrhage (Lindqvist *et al*., 2001). On the other hand, FVL mutation in females might also be associated with negative outcomes of reproduction such as recurrent abortion, pre-eclampsia, prematurity and small-for-gestational-age neonates (De Groot *et al*., 1999; Rai *et al*., 2001; Morrison *et al*., 2002; Hundsdoerfer *et al*., 2003; Pauer *et al*., 2003; Krabbendam *et al*., 2005). As the inheritance pattern of FVL can best be described as co-dominant, the status of both maternal and paternal FVL is likely to be of significance. FVL status in males in relation to reproduction has not been investigated to date. Although it seems unlikely that FVL status *per se* would influence male fertility, no published data on this topic are available. Whether the FVL status of the embryo as such has any influence on reproductive success remains to be clarified.

A high fecundity rate (shorter time to a desired pregnancy) may reflect implantation success. However, in a recent study concerning only females that had suffered venous thrombosis, FVL was not associated with a change in fecundity (van Dunné *et al*., 2005). A population of males and females with their fertile years in an era in which fertility control was minimal

appears suitable for the analysis of the influence of FVL on fertility and fecundity. In this study, we assess fertility and fecundity in a large cohort of subjects born in the late nineteenth and early twentieth centuries, who were of childbearing age in a time where modern contraceptive methods were unavailable.

MATERIALS AND METHODS

The Leiden 85-Plus Study consists of two separate cohorts. A detailed description of both cohorts has been presented elsewhere (Van Aken *et al*., 2002). In short, subjects of the first cohort were enrolled between December 1986 and March 1989. During that period, a total of 977 inhabitants of Leiden, The Netherlands, who were aged 85 and over were included. A second cohort of 85-year-olds, consisting of 599 subjects, was enrolled between September 1997 and September 1999. There were no selection criteria for health or demographics in either cohort. Of all subjects, a blood sample was obtained. DNA was available for an unselected sample of 660 subjects in the first cohort (68%) where the FVL mutation could be determined in 653 subjects. In the second cohort, cell material was available for 561 subjects (94%) where the FVL mutation could be determined in 555 subjects. The Registry of Births, Deaths, and Marriages of the municipality of Leiden and the Central Bureau of Genealogy (CBG), The Netherlands, provided the date of birth, date of marriage(s) and birth dates of children of all study participants. The CBG is the major documentation and information centre for family history and heraldry in the Netherlands. For 32 subjects, there was insufficient information available on their marital history or their number or dates of birth of progeny. Hence, complete information was available for 1176 subjects. The Leiden University Medical Centre's medical ethical committee approved the protocol for both cohorts.

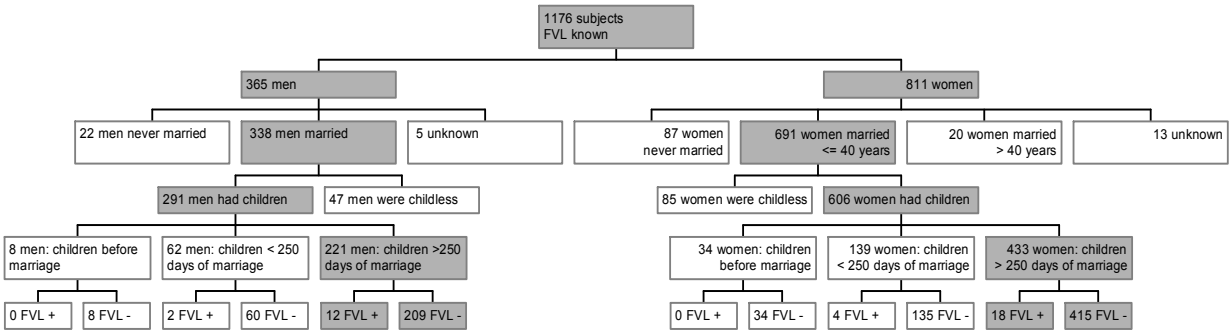

Figure 1. Selection of subjects for the Leiden 85-Plus Study with known factor V Leiden (FVL) status.

Fecundity was defined as the calculated time interval between the date of (first) marriage and the date of birth of the firstborn child. This concept of delay from marriage to the first birth has previously been defined as 'effective fecundability' (Leridon, 1977). This effective fecundability was arbitrarily divided into groups according to probable conception time. If the conception had taken place within the first 3 months of marriage, it can be assumed that these children were most likely born within 250 and 371 days of the marriage date. This was calculated by adding 3 months (91 days) to the median duration of a term pregnancy (280 days). Likewise, if conception had occurred within 6 months of marriage, the children would most likely be born between 250 and 463 days after marriage. For conception within 12 months of marriage, the date of birth was assumed to be within 250 and 645 days of marriage. To minimize the selection of pregnancies conceived before marriage, children born before marriage or within the first 36 weeks (250 days) of marriage were excluded from analysis. Women with an age beyond 40 at the time of their marriage were excluded from further analysis due to the rapid decline of fertility and fecundity that can be expected from that age onwards. Figure 1 illustrates the flow chart of the participating subjects.

For the present study, the FVL analysis was done at the same moment in time for all available 1176 stored blood samples. Assessment of the FVL mutation in DNA was determined by PCR and *Mnl*I restriction digestion as described elsewhere (Bertina *et al*., 1994; Heijmans *et al*., 1998).

The two groups were compared using Student's *t*-test for continuous variables and Pearson's chi-square test for categorical variables with Fisher's exact test applied when the expected frequencies were <5. All tests were two-tailed.

Table I. Characteristics of 338 married male and 691 female subjects married at or before 40 years of age according to their factor V Leiden carrier status.

	Men		**Women**	
	FVL +	**FVL -**	**FVL +**	**FVL -**
	(n =15)	**(n =323)**	**(n =26)**	**(n =665)**
Age at marriage	26 (22-39)	26 (18-61)	25.5 (19-39)	24 (17-40)
Age at birth first-born	27.5 (23-48)	28 (17-56)	27.5 (20-40)	26 (13-40)
Year of birth first-born	1935 ('21-'61)	1936 ('12-'59)	1935 ('18-'53)	1931 ('10-'54)
Number of children	2 (1-5)	3 (1-12)	2 (1-8)	3 (1-11)
Childless (%)	2 (13)	47 (15)	4 (15)	82 (12)

Values are median (range) or n (%). FVL = Factor V Leiden.

RESULTS

The 1176 subjects included in this analysis comprised 365 men (31%) and 811 women (69%). The year of birth ranged from 1883 to 1914. The FVL mutation was present in 18 men (4.9%) and 33 women (4.1%). All were heterozygous for FVL. There were no homozygotes. A total of 338 (93%) of the male subjects had been married at least once and 691 (85%) of the female subjects were married at or before the age of 40. Two hundred and ninety-one of the married men had children, 8 (3%) men had a child before marriage (all non-carriers) and 62 (21%) men had their firstborn child within 250 days of marriage and were therefore excluded (two were FVL carriers). Six hundred and six of the women married at or before the age of 40 had children, 34 (6%) had a child before marriage (all non-carriers). In 139 (23%) women the firstborn was recorded within the first 250 days of marriage (four FVL carriers) and therefore excluded under the assumption that conception had taken place before marriage. The year of birth of the firstborn child ranged from 1912 to 1961 in male subjects and from 1910 to 1954

in female subjects. The number of children was unrelated to the presence of the FVL mutation (Table I). A similar number of marriages remained childless in FVL carriers and non-carriers regardless of gender (Table I).

Table II. Assumed conception time calculated for the births occurring more than 250 days after marriage for the 221 married men (12 FVL+ and 209 FVL–) and 433 women (18 FVL+ and 415 FVL–) married at or before 40 years old

	Men		
	FVL + (%)	FVL – (%)	Relative risk (95% CI)
Conception ≤ 3 months of marriage	8 (67)	40 (19)	3.5 (2.1-5.7)
Conception > 3 months of marriage	4 (33)	169 (81)	
Conception ≤ 6 months of marriage	9 (75)	72 (34)	2.2 (1.5-3.2)
Conception > 6 months of marriage	3 (25)	137 (66)	
Conception ≤ 12 months of marriage	9 (75)	108 (52)	1.5 (1.0-2.1)
Conception > 12 months of marriage	3 (25)	101 (48)	
	Women		
	FVL + (%)	FVL – (%)	Relative risk (95% CI)
Conception ≤ 3 months of marriage	4 (22)	112 (27)	0.8 (0.3-2.0)
Conception > 3 months of marriage	14 (78)	303 (73)	
Conception ≤ 6 months of marriage	7 (39)	171 (41)	0.9 (0.5-1.7)
Conception > 6 months of marriage	11 (61)	244 (59)	
Conception ≤ 12 months of marriage	10 (56)	233 (56)	1.0 (0.6-1.5)
Conception > 12 months of marriage	8 (44)	182 (44)	

CI: confidence interval; Values are *n* (%).
Conception ≤3 months: birth of firstborn child within 250 and 371 days of marriage;
Conception ≤6 months: birth of firstborn child within 250 and 463 days of marriage;
Conception ≤12 months: birth of firstborn child within 250 and 645 days of marriage.

Table II presents the assumed conception time of married men and women dependent on their FVL status. In female subjects, FVL carriers had similar fecundity rates compared with non-

carriers [relative risk (RR), 0.8; 95% confidence interval (CI), 0.3–2.0; $P = 0.79$]. Male FVL carriers had a 3.5-fold (95% CI, 2.1–5.7; $P < 0.001$) increase in the probability of conception of a child within the first 3 months of marriage compared to non-carriers. Within 6 months of marriage, the results remained similar (RR, 2.2; 95% CI, 1.5–3.2; $P = 0.01$). In an additional analysis, with all births from the first day of marriage onwards included, without the 250-day threshold, the results for males remained significant (RR, 1.9; 95% CI, 1.3–2.8; $P = 0.01$ at 3 months and RR, 1.6; 95% CI, 1.2–2.2; $P = 0.03$) at 6 months.

DISCUSSION

In the present study of 1029 married male and female subjects born between 1883 and 1914, fecundity in females was unrelated to FVL status. In males, there was an unexpected, but highly statistically significant finding of an increased fecundity (shorter time period between marriage and firstborn child) in FVL carriers compared with non-carriers. There was no association between FVL mutation and fertility or family size.

Heterozygous FVL mutation was found in 4.3% of subjects, similar in male and females. This is comparable with earlier published data on FVL prevalence in the Dutch population (Rees *et al*., 1995). There were no individuals homozygous for factor V Leiden, which is within expected numbers as the population prevalence is 0.1%.

Fecundity in females was comparable in FVL carriers and non-carriers. The current study only comprised completed pregnancies; there was no information available on pregnancies ending in miscarriage or fetal loss. Female FVL carriers may have had higher rates of miscarriages or fetal loss, reducing the amount of children born within the first year and lowering fecundity rates masking an effect of FVL on embryo implantation in females. This seems unlikely, however, as an earlier study found that the number of reported miscarriages was similar in FVL carriers compared with non-carriers (van Dunné *et al*., 2005).

In male subjects, FVL carriers had a significantly increased fecundity compared with non-carriers. An explanation for these findings in the male population can only be speculative. The results may be real, due to a chance finding or due to a selection bias. In selecting only the

births from 250 days after marriage (assumed to be conceived after the marriage date), a bias may occur in selecting the less-fertile couples (Sallmen *et al*., 2005). The couples that get married due to an unintended pregnancy will have their babies with a shorter interval after marriage, they will be excluded and presumably, they are the most fertile. However, FVL carriers were evenly distributed in subjects with births that occurred before the first 250 days of marriage and beyond that time in both males and females. Moreover, with all the births from the first day of marriage included, the results remained similar. Although elderly subjects (over 85 years of age) were selected, the FVL prevalence has been reported to remain stable at this age, and it does not affect population mortality (Heijmans *et al*., 1998). Hypothetically, the location of the *FVL* gene could be in the proximity of an unknown, male-fertility gene elevating the risk of a mutation in that gene, resulting in an increase in sperm numbers or motility. An analogous phenomenon is seen in cystic fibrosis (CF) where mutations in *CF* genes cause typical CF symptoms but also cause congenital bilateral absence of the vas deference and infertility in 99% of males with CF (Lissens and Liebaers, 1997). Whether FVL has any effect on sperm quality or quantity has never been investigated. Furthermore, FVL may have a positive effect on implantation (Majerus, 1994) by way of the inheritance of the paternal FVL mutation by the embryo. An FVL-positive embryo may have a higher likelihood of implantation in an FVL-negative mother. Indeed, a few small studies have reported a higher-than-expected FVL mutation rate in infants born to mothers in various (normal) control groups compared with the reported prevalence of FVL mutation in the normal population (Currie *et al*., 2002; Schlembach *et al*., 2003). Further research is required to distinguish whether not only maternal FVL status but also paternal status and subsequently the embryo is of significance for reproductive success.

In the present study, with births ranging from 1918 to 1954, 25% of subjects had a child within the first year of marriage; therefore their calculated conception time was within the first 3 months of marriage. Fifty-five percentage of subjects had their first child within 21 months of marriage, corresponding with a calculated conception time within the first 12 months of marriage. In recent times, conception rates are reported considerably higher. From 1961 to 1993, fecundity rates in Britain increased significantly for both men and women. Cumulative pregnancy rates for 1961 were reported as 56% at 3 months and 79% at 12 months. For 1993, these figures were 66% and 90%, respectively (Joffe, 2000). In recent

prospective studies, clinical pregnancy rates were as high as 65–70% in the first three cycles and 81–90% in the first six cycles (Gnoth *et al*., 2003; Wang *et al*., 2003). An explanation for this increase in fecundity may be a change in general behaviour due to more knowledge about fertility and therefore a more optimal timing of intercourse. The readily available and reliable contraception nowadays will enhance family planning with an increased focus to having a child at a specific time. Furthermore, the recent prospective studies include pregnancies ending in a miscarriage, which was not available in the present study.

The current study has some limitations. All reproductive information was acquired from registries; therefore, all conception times and fecundity rates were calculated. It is possible that not all pregnancies ending in a death of the fetus at term were reported. Whether FVL carriers may have had more premature births ending in neonatal deaths remains speculative. The selected cohort was set in a time represented by minimal fertility control and no modern contraceptive methods. We have assumed that starting a family as soon as a marriage was celebrated was desired. The circumstance of the subjects at the time of their marriage is unknown. Any significant illnesses or availability of either partner in the first year after marriage is unknown. Other factors interfering with fecundity such as sperm count, regularity of menstrual cycle and frequency of intercourse are unknown. However, it is not likely that FVL itself will interfere with these factors. Male FVL carriers were not younger at marriage, and their spouses had a similar age at marriage (median 25 years old, range 22–37) to the females included in our cohort.

In conclusion, we found that the FVL mutation increases fecundity in males, with a shorter interval between marriage and birth of the first child in an era prior to modern contraceptive use. This was not found in females. Possible explanations are that FVL increases male fertility, linking the *FVL* gene to a fertility gene that may potentially increase sperm count or motility. Another explanation might be that the presence of the FVL mutation in an embryo increases the implantation rate in an FVL-negative mother. The ‘antagonistic pleiotropy’ theory (Williams, 1957) regarding the fixation of alleles with positive effects upon fitness early in life with deleterious effects late in life may well apply to the FVL mutation.

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7

General discussion

GENERAL DISCUSSION

1. GENETIC FACTORS AND HUMAN REPRODUCTION

The two genetic factors highlighted in this thesis are considered to interfere with human reproduction through different pathways. The first pathway studied is the innate immune response by way of cytokines that acts through regulation of an inflammatory process. The process of inflammation is known to induce angiogenesis[1]. Angiogenesis is the formation of new blood vessels from pre-existing ones, and is acknowledged to play an essential role in the process of embryo implantation[2;3] and cytokines are considered to be involved in this process[4]. The cytokine interleukin-10 (IL10) is studied in detail in this thesis as it is thought to have an important role in pregnancy[5;6]. IL10 tempers pro-inflammatory cytokines and induces a shift towards a more anti-inflammatory immune response, creating a favourable balance for the acceptance of the semi-allogenic embryo in the maternal uterus[6].

The second pathway studied is by way of increased general coagulability due to the factor V Leiden mutation. Coagulation is a necessary step during the implantation process considering that the blastocyst invades the trophoblast with numerous capillaries[2]. If excess bleeding would occur the fate of the embryo may be jeopardised. In this process the factor V Leiden status of the mother and the embryo are separate entities. Possibly the Factor V Leiden mutation in an embryo alters the chance of implantation during the very early phases of life.

2. TRADE OFF?

It is questioned whether the molecular mechanisms that are described in this thesis would support the disposable soma theory; i.e. would fit the trade off between reproductive success and longevity. Regulation of immunity is an obvious candidate because the adverse conditions in our (natural) habitat necessitate large investments to fight infections and therefore reach (post)reproductive age. Cytokines are critical signalling molecules. Tumor necrosis factor-α (TNFα) for instance will initiate an inflammatory response to fight infection. Regulatory signals from IL10 taper the inflammatory response and prevent collateral damage after the infection has been overcome. It has now been shown that the production capacity of these cytokines appears to be under tight genetic control[7].

In an earlier study the levels of IL10 and TNFα were studied in first-degree relatives of patients who suffered meningococcal disease, an infection that is widely present in Africa and occasionally surfaces in developed countries[8]. It was therefore assumed that families of those patients who had died would have a distinct pattern of cytokine activity. Almost without exception, the level of pro-inflammatory TNFα in all of these cases was low, and the level of the anti-inflammatory IL10 was high. The interpretation of these data was that subjects with an innate propensity towards anti-inflammatory responses are at an increased risk of death from infection. IL10 responsiveness was furthermore found to be reduced at old age. Innate IL10 responsiveness was significantly reduced in subjects over 85 years old compared to both 14-40 year olds and 41-75 year olds. When LPS-induced cytokine levels were compared between a random selected subgroup of 85-year old and younger control donors, the 85-year old study participants produced lower levels IL10 (16). Whether the various IL-10 promoter alleles are of influence on longevity remains inconclusive. An Italian study found that the *IL-10* gene SNP -1082G-A allele had a significant influence on the attainment of longevity in men[9] this in contrast to a Finnish population study where IL-10 promoter alleles and haplotype frequencies were not different between nonagenarians and controls[10].

In contrast with fighting infection, which requires a strong inflammatory host response, reproductive success depends on a tolerant immune response[6], though it is possibly not essential[11]. The effect of IL10 in early pregnancy can be explained in two ways. Firstly, the tissue antigens of an embryo are partly of paternal origin, therefore at the fetal−maternal interface immune reactions must be suppressed to allow pregnancy to be accepted and to proceed. Inhibition of the pro-inflammatory (Th-1) immunity, for instance as a result of the immuno regulation of IL10, is considered necessary for the acceptance of the semi-allogenic fetus[5;12;13]. Secondly, the effect of IL10 may be explained by inhibiting the local inflammatory reaction. This inflammatory reaction has been shown to be important for successful pregnancy in mice[14]. Possibly very high levels of IL10 decrease local inflammation possibly reducing the chance of successful implantation. The limits of optimal IL10 levels are probably regulated. However this may indicate that the effects of differences in genetic dissimilarity in IL10 production will initially have an effect on the immune system rather than on the minute amounts of inflammation necessary to induce a vascular response.

In this thesis we have shown that the cytokine profile of women with impaired fertility (defined as having at least three consecutive spontaneous miscarriages) is markedly different when compared to the profiles of women of normal fecundity[15]. The data on the cytokine profiles help to elucidate two phenomena. First, they can explain why British aristocrats (see introduction), who lived longer, were less likely to have successful pregnancies. Their innate immune system favoured resistance to infection but at the same time prevented pregnancy from proceeding, a trade-off that was even stronger in times when the environmental conditions were relatively poor. Second, it explains why a genotype associated with impaired fertility might have persisted in spite of its obvious disadvantage with regard to evolutionary fitness. Selection for resistance to infection is traded against selection for fertility, resulting in a compromise that is optimal for the fitness of the species in a specific environment.

The molecular mechanisms that can explain the trade off between reproductive success and longevity are not confined to the regulators of immunity. In this thesis the factor V Leiden mutation has been postulated to facilitate the process of embryo implantation. It may be beneficial for an embryo to possess this gene mutation early in life, for instance increasing the chance of implantation, but on the other hand increasing the chance of a possibly life threatening thrombotic event later in life. However, factor V Leiden has been shown to have the same prevalence in old age as early in adult life[16]. Possibly the increased therapeutic options available in prophylaxis and treatment of thrombotic events play a role in the similar prevalence found at high age. Whether a 'trade off' in this sense exists remains unclear.

3. CYTOKINES AND COAGULATION IN PREGNANCY

Inflammation and coagulation are important factors in pregnancy. Normal pregnancy is both an acquired hypercoagulable and inflammatory state[17;18]. A clear association between factor V Leiden and cytokine production (in particular IL10 and TNFα) has not been observed so far[19]. Effects of other cytokines on coagulation and vice versa have been stated in the literature. First, a direct effect of cytokines on coagulation is proposed by up-regulating fibrinogen-like protein 2 (fgl2) prothrombinase[20]. Fgl2 is a glycoprotein that is capable of directly cleaving prothrombin to thrombin leading to fibrin deposition[20]. Increased levels of Th1 cytokines (i.e.

TNFα) have been shown to activate coagulation via up-regulating fgl2 [21]. Fgl2 is thought to play a role in spontaneous miscarriages[22;23].

Second, a pathway is proposed through the presence of circulating pro-coagulant microparticles (cytoplasmatic components and membrane-derived elements from various cells) found in pregnancy[24]. These microparticles also act as potent pro-inflammatory agents[25]. The concentrations of these pro-coagulant microparticles are increased in the peripheral circulation of both women with both early and late miscarriages compared to women with normal pregnancies[25;26].

Third, a possible link that a connection exists between coagulation and inflammation may be that treatment with anticoagulant medications, such as heparin may not only have properties regarding anticoagulation, but also may have other means of interfering with pregnancy[27;28]. Interactions between heparin and cytokines have been published, for instance inhibiting the anti-inflammatory cytokine IL-8 in rats[29] but also inhibiting the inflammatory TNFα in a mouse model[30]. It has furthermore been suggested that heparin interferes with the adhesion of the blastocyst to the endometrial epithelium and the subsequent invasion[31]. Further evidence of this relation between heparin and pregnancy remains to be verified.

4. TH-1/TH-2 PARADIGM IN PREGNANCY?

As been stated previously, pregnancy has been classified as a Th-2 mediated phenomenon where suppression of pro-inflammatory cytokines in the decidua is assumed to be important for successful placentation[5;32]. It has been suggested that this Th-1/Th-2 hypothesis represents an oversimplification of the situation[33;34]. Cytokines are produced not only by T-helper cells (Th-1 or Th-2 cells) but also by cells other than immune cells, including macrophages, epithelial and stromal cells of the endometrium and decidual and cytotrophoblast cells of the placenta[35]. Also early IVF embryos have been found to produce various cytokines including IL10[36]. Moreover, it is acknowledged that cytokines have overlapping functions and can be both anti- as pro-inflammatory[37]. As a result it seems less probable that there is a strict separation between a Th-1 and Th-2 immune response and a more complex core is likely to exist.

However, in principle, the results in this thesis support the original hypothesis that enhanced secretion of anti-inflammatory (Th-2) cytokines is a characteristic of a normal physiologic pregnancy[5]. We found that women with a high fecundability (who were pregnant within 3 months) were 16 times more likely to have a high IL10 and low TNFα responsiveness compared to women who suffered recurrent miscarriages. Also in women with rheumatoid arthritis (a Th-1 mediated disease) who had suffered a miscarriage had higher likelihood of developing a more severe disease measured by a more severe joint damage in the first two years of presenting. At a genetic level only one IL10 SNP was found to be related to fertility: the IL10 -2849 AA genotype increased the likelihood of remaining childless in a female married population and increased the time interval between marriage and birth of the first-born child. The relation between non-exon SNPs and gene function is however difficult to prove by either biochemical or molecular biological methods. This is because it is unknown which stimulus eventually leads to the increased IL10 secretion in pregnancy. Given the fact that little knowledge is present on the exact biological process it is not known which transcriptions factors are involved in addition to whether a change in the transcription factor binding site is relevant. Thus we examined whether IL10 -A2849G was merely a tag of a haplotype or whether it alone was the best predictor of fertility characteristics. Indeed, no relation between the IL10 haplotypes and fertility or fecundity could be found, although it was generated by a limited number of SNPs. We therefore proposed that the IL10 -A2849G SNP is related to an altered gene function in regard to fertility. The low IL10 responsiveness that is particularly found in relation to the IL10 –2849 AA genotype may be crucial in explaining these results[15;38]. A low IL10 responsiveness may reduce the chance of developing a successful pregnancy.

It is beyond doubt that the mechanism stated above is just a small part of a far bigger story. IL10, as a Th-2 cytokine is beneficial for a successful pregnancy. However, not only Th-2 but also a number of Th-1 cytokines seem crucial for successful implantation of the blastocyst. This has been indicated in animal studies. Interestingly, IL10 knock-out mice have been shown to have normal reproduction results[11;39] contrary to general inflammatory knock-out mice that all resulted in implantation failure[40;41]. Maybe we should rephrase Wegmann's hypothesis of pregnancy as a Th-2 phenomenon into 'pregnancy is a Th-2 phenomenon that cannot occur successfully without Th-1 components taking place'.

5. A TOO EASY ACCEPTANCE OF A PREGNANCY?

Theoretically, miscarriages can be seen as a safety net to filter out a chromosomal or morphological abnormal embryo. If miscarriages would not occur, many more severely abnormal children would be born, most probably not surviving birth[42]. In this thesis a reduced number of first trimester miscarriages was found in relation to maternal factor V Leiden carriership, without altering the miscarriage rate overall. An explanation of this finding remains to be elucidated. One suggestion may be that as factor V Leiden increases coagulation, it may well interfere with coagulation locally at the site of implantation of the blastocyst in the endometrium. The blastocyst is known to invade blood vessels as it penetrates into the luminal epithelium of the endometrium forming the trophoblast that contains multiple cavities with maternal blood[43]. It is possible that a decreased likelihood of bleeding may reduce the chance of the early pregnancy to fail (i.e. the blood loss itself as a cause of the pregnancy failure, not as a result). Factor V Leiden might increase the chance of implantation due to the enhanced coagulability, conceivably by decreasing the amount of blood loss occurring at implantation[44]. Possibly, this may increase the chance of a pregnancy to continue regardless of the existence of a chromosomal or morphological abnormality in the embryo. This would assume that the abnormal embryo will miscarry later in pregnancy, in a next step of Mother Nature's safety net. Another theory may be that the high coagulability of a factor V Leiden carrier may increase the anchoring of the embryo to the endometrium. This higher coagulability may also increase the likelihood of thrombosis occurring in the trophoblast or early placenta, resulting in a failure of the pregnancy.

This assumption however does not completely cover the findings we made in this thesis. Paternal factor V Leiden carrier ship was found to be important, independent to the maternal factor V Leiden status. An increased fecundity (a shorter time interval between unprotected intercourse and the occurrence of pregnancy) was found if the father carried the factor V Leiden mutation, but was not found if the mother carried the mutation. It was opted that the factor V Leiden status of the fetus (inherited by the father) is significant in increasing the likelihood of embryo implantation. This remains a hypothesis, as the factor V Leiden status of the child was not retrievable. Furthermore it is unknown whether or how a blastocyst exposes

the factor V Leiden gene and if this has any effect on implantation. Maybe a factor V Leiden positive embryo has a higher likelihood to adhere or anchor to the endometrium increasing the chance of implantation; however this remains an unsubstantiated thought. Given the fact that this finding did not come from a prior defined hypothesis, it may also be a spurious association which will need further confirmation in additional studies.

This leads to a different hypothesis altogether. Possibly women suffering recurrent abortions (and perhaps even women with a decreased fecundity) have a low rather than a high threshold of accepting a pregnancy. Perhaps they have a higher likelihood of not only accepting normal embryos but also the abnormal ones that will eventually miscarry. Previously it has been stated that early (preclinical) pregnancy loss rather than failure of conception may be the principal cause for the relatively low fecundity observed in humans[45]. Per cycle the chance of fertilisation is about 70-80% however about 60% of these conceptions will miscarry, mainly before a clinical pregnancy can de diagnosed[45]. Perhaps therefore, we need to seek more in the direction of a too easy acceptance of a pregnancy. Maybe women with unexplained recurrent miscarriages have an increased tendency to accept every conception even if the early embryo has abnormalities (chromosomal or morphological) and is therefore wrongfully accepted. The pregnancy is not 'filtered' in the very early stages and can progress beyond the menstrual date when a clinical pregnancy can be diagnosed. However, as the embryo is abnormal, the pregnancy will most likely be rejected in the following weeks as the embryo will fail to develop and a miscarriage will occur. If a woman has a tendency to accept an embryo too easily, it would explain the occurrence of some recurring miscarriages and may be seen as a natural protection for having healthy offspring. One way of testing this hypothesis is to analyse all early pregnancies not only on chromosomal abnormalities but also to check for morphological abnormalities.

This hypothesis of an increased early acceptance of a pregnancy may be used not only for factor V Leiden but also for an excessive Th-2 cytokine profile. For instance diseases that are known to be Th-2 mediated with an increased IL10 production, like systemic lupus erythematosis (SLE), an increase in miscarriages is seen[46;47]. Possibly the less optimal embryo's are accepted more readily in these phonotypical Th-2 mediated diseases, subsequently ending in more miscarriages. It may well be that there is an optimal basal IL10

production that is most favourable for a successful pregnancy. A low basal IL10 production may not be enough to inhibit the Th-1 resonse well enough for the acceptance of the semi-allogenic fetus, but a high basal IL10 production may be detrimental in reducing the necessary inflammatory reaction needed for angiogenesis during the blastocyst invasion[3;40;41]. Further research using conditional IL10 knock-out mice may be an option.

6. CLINICAL IMPLICATIONS AND FUTURE RESEARCH

It is too early to translate the findings written in this thesis towards the day-to-day clinical care. It is not time yet to administer cytokines to pregnant women, as the effect will be systemic, with unknown effects on the developing fetus. Moreover, the effect of one cytokine will be diverse as it will trigger a whole cascade of other cytokines to be stimulated or inhibited, with unknown results for the pregnancy. The results found regarding factor V Leiden are also preliminary and do not alter clinical care at his stage. It may inform the clinician however, that factor V Leiden carrier ship in mother or fetus may have different consequences, and that paternal influences may be significant.

Future research should be focused on the pro-inflammatory and anti-inflammatory cytokine profile from the conception and implantation onward, with an emphasis on genetic aspects of these cytokines. Not only the maternal profile but to include the paternal profile and test the fetal profile (at birth or if possible at time of miscarriage). Concerning IL10 specifically, it would be interesting to distinguish whether different levels of IL10 mediate different effects. An interesting hypothesis may be whether a low basal rate of IL10 is necessary to increase the likelihood of implantation and whether or not the embryo is rejected is determined by differences of IL10 production at a much higher basal level.

With regard to research on miscarriages, both in relation to factor V Leiden and cytokines, it will be important to assess if the embryo/fetus was (ab)normal. An abnormal embryo may miscarry due to a number of reasons, and the balance of pro- and anti-inflammatory cytokines might be different than when a normal embryo miscarries. The assessment of the morphology of the fetus can be done by 2D or 3D ultrasound[48] or embryoscopy after a miscarriage has been diagnosed[49]. Furthermore, karyotyping of the fetal products will be important to assess if

the embryo had numerical chromosome abnormalities. Similar research could be done in fetuses that are aborted without a medical indication (abortus provoatus) and may be used as a control group.

A too 'hostile' maternal environment, but also a too 'friendly' maternal environment may induce an increase in incidence of miscarriages. These observations indicate that the processes involved in early pregnancy need to be further delineated. Subsequently it is necessary to identify the rate of limiting steps in normal physiology to understand the disturbances in fertility in the human population. Ideally this is done in animal studies first, followed by genetic association studies to find out which of these processes are rate limiting. Finally this could lead to designs of intervention trials in which patients with repeated miscarriages can be treated. Ultimately this could lead to less suffering of couples experiencing early pregnancy problems.

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8

Summary

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SUMMARY

In **Chapter 1** the background and scope of the studies presented in this thesis is given. Genetic factors play an important role in the regulation of human life span but the exact pathways remain to be elucidated. It is an intriguing idea that these pathways are interrelated with the regulation of human reproduction. The view is that the chance of identifying the critical genes in either or both of these characteristics is likely to be increased when studying both characteristics at the same time.

Human reproduction is a process that appears remarkably inefficient. It nevertheless produces very good outcomes as the vast majority of ongoing pregnancies will result in the birth of a healthy child. A longer time to pregnancy (fecundity) and miscarriages is an inevitable by-product of such a process. Overall, the average fecundity rate per menstrual cycle in humans is about 15-20%. A miscarriage is the premature expulsion of a nonviable fetus from the uterus, usually before the middle of the second trimester of gestation. Only 30-50% of all conceptions result in a live birth. There are many different causes for a pregnancy to fail. It is feasible that a genetic predominance or an innate mechanism is one of them. It is most likely to find this in couples with a decreased fecundability or suffering (recurrent) miscarriages, maybe more so when it can be liked to another genetic predominant feature like longevity. Longevity refers to the length or duration of life and living a long life beyond the norm for the species This link between reproduction and longevity was tested for two phenomena, the innate Th-1/Th-2 immune response, mainly through Interleukin 10 (IL10) and Tumour Necrosis Factor α (TNFα) and a factor in the clotting cascade: the factor V Leiden (FVL) mutation.

In **Chapter 2** it is argued that an innate cytokine profile supportive of Th1-type T cells favors survival of infectious diseases (with longevity as the ultimate), but women with this profile are found less likely to have successful pregnancies (progeny). During evolution selection for fertility (a cytokine profile favoring Th-2 type T cells) is optimized with the selection for survival (a cytokine profile that favors the development of Th-1 type T cells). The probability of a normal fertility increased more than 10-fold when the innate cytokine profile was characterized by high IL-10 (Th-2) and low TNF-α (Th-1) responsiveness, compared to women who had experienced recurrent miscarriages.

In **Chapter 3** different IL10 promoter polymorphisms (Th-1) at position –2849, -1082 and –592 were analysed in association with fertility and fecundity in both male and female subjects (1116 total) enrolled in the Leiden 85-Plus Study. The -2849 A allele has been found to be associated with a decreased IL10 responsiveness. We found that fertility was decreased in association with the –2849 A allele in females compared to the female G allele carriers (OR: 2.2, 95% CI: 1.2-4.2). Fecundability was decreased in association with the –2849 A allele in females compared to female G allele carriers (OR: 0.2, 95% CI: 0.04-0.7). This suggests that the IL10 –2849 AA genotype is associated with a decreased fertility and fecundity in females, possibly due to the lower expected IL10 responsiveness. In male subjects no such association was observed.

When the immune system mistakes 'self' tissues for 'non-self' and mounts an inappropriate attack, it can result in an autoimmune disease. Rheumatoid arthritis (RA) and is an example of an autoimmune disease that predominantly occurs in females. RA is a Th-1 mediated disease where cytokines are important mediators, these cytokines also play a vital role in the acceptance and maintenance of pregnancy. In **Chapter 4** it is investigated whether the reproductive history prior to disease onset is predictive of the severity of RA in women. A cohort of 113 female patients attended a special early arthritis clinic (EAC) for their newly diagnosed RA. A structured questionnaire was taken concerning their reproductive history and the joint damage was measured by sequential X-rays of hands and feet, using the modified Sharp score. Fecundity was comparable to the general population data and it did not reflect to the extent of joint damage over time. The miscarriage rate was in these RA patients was 15% per pregnancy, also comparable to population figures (12-15%). However, a significant increase in joint damage over a 2 year follow-up was found in RA patients who had experienced at least one miscarriage compared to patients who never had a miscarriage in the past (mean modified Sharp score at 2 years 24 (95% CI:15-32) and 16 (95% CI:10-23) respectively). At baseline the Sharp scores were similar in both subgroups. The results may indicate that the phenotype of joint destruction is associated with the phenotype of reported miscarriages, suggesting common genetic risk factors for each of these two traits, possibly through the innate Th-1/Th-2 phenotype.

Chapter 5 considers a factor of the clotting cascade in relation to fertility and fecundity. Factor V Leiden (FVL) is a point mutation in the factor V gene (Arg506Gln) which is prevalent in 3-10% of Caucasians. FVL has a disadvantage in adult lifetime with a 7-fold increase in incidence of deep vein thrombosis. Pregnancy in general is a hypercoagulable state. Nevertheless, positive effects of FVL have been postulated. An improved implantation rate in intra-cytoplasmatic sperm injection (ICSI) pregnancies was previously reported if either the mother and/or the fetus carried the FVL mutation. FVL mutation is therefore hypothesized to support embryo implantation. In 115 female venous thrombosis (VT) patients with FVL mutation and 230 age-matched female VT patients without FVL, the reproductive history was queried. We found that fecundity was unaffected by FVL carrier status. With reference to first trimester miscarriage, this was reported less frequent among FVL carriers (46%) than among non-carriers (95%) (RR 0.5, 95% CI: 0.3–0.9). The overall miscarriage proportion was however not influenced by the FVL status. These results may suggest that FVL offers a protective effect in early pregnancy and that the miscarriage of embryos with poor viability in FVL carriers is postponed until the second trimester. Possibly an increased local thrombotic tendency may increase the likelihood of implantation of a blastocyst (embryo).

With this in mind we analysed a cohort of 1029 married male and female subjects born between 1883 and 1914 who were enrolled in the Leiden 85-Plus Study. This is reported in **Chapter 6**. The Registry of Births, Deaths and Marriages Leiden provided the dates of birth, marriage and birth(s) of children. No information on miscarriages could be obtained. In females, fertility and fecundity was unrelated to the FVL status. In males, there was an unexpected, but highly statistically significant finding of an increased fecundity (shorter time period between marriage and firstborn child) in FVL carriers compared with non-carriers (RR: 3.5; 95% CI: 2.1–5.7). There was no association between FVL mutation and fertility or family size in males or females. Possible explanations of this finding can only be speculative; FVL may increase male fertility, linking the factor V Leiden gene to a fertility gene that may potentially increase sperm count or motility, or more likely, that the presence of FVL mutation in an embryo may increase the implantation rate in a FVL negative mother.

In **Chapter 7** the combined results of the studies are discussed in a broader perspective.

SAMENVATTING

Hoofdstuk 1 bespreekt de achtergrond en de reikwijdte van dit proefschrift. Genetische factoren spelen een belangrijke rol bij het reguleren van de duur van een mensenleven. De precieze manier waarop dit gebeurt is echter nog onduidelijk. Het is een interessant gegeven dat deze manier is gerelateerd aan de regulering van de menselijke voortplanting. De kans om de essentiële genen te identificeren in een of beide van deze onderwerpen wordt waarschijnlijk vergroot als beide tegelijkertijd worden bestudeerd.

De menselijke voortplanting lijkt een opvallend inefficiënt proces. Daar staat tegenover dat de overgrote meerderheid van zwangerschappen resulteert in een gezond kind. Een langere duur tot de zwangerschap (fecunditeit) en miskramen zijn een onvermijdelijk bijproduct van dit proces. In het algemeen is de gemiddelde fecunditeit per menstruatiecyclus rond de 15 á 20%. Een miskraam is de voortijdige uitdrijving van een niet levensvatbare foetus uit de baarmoeder, meestal voor het midden van het tweede trimester van de zwangerschap. Slechts 30 á 50% van alle concepties leiden daadwerkelijk tot een levend geboren baby. Er zijn vele oorzaken waarom een zwangerschap kan mislukken. Het is mogelijk dat een genetische predominantie of een aangeboren mechanisme daar één van is. Het is het meest waarschijnlijk dat dit te traceerbaar is bij koppels met een verminderde fecunditeit of bij wie herhaalde miskramen voorkomen, mogelijk met name waar het kan worden vergeleken met een ander genetisch predominant gegeven zoals langlevendheid. Onder langlevendheid wordt verstaan de lengte of duur van leven, of ook wel een leven dat langer duurt dan voor de soort gebruikelijk is. Dit verband tussen voortplanting en langlevendheid is op twee gebieden getest: de aangeboren Th-1/Th-2 immuun respons, voornamelijk door Interleukine 10 (IL-10) en Tumor Necrosis Factor α (TNFα), en een factor in de bloedstollingcascade, te weten de factor V Leiden (FVL) mutatie.

In **Hoofdstuk 2** wordt beargumenteerd dat een aangeboren cytokine profiel dat het Th-1 type T cellen ondersteunt, de kans op overleving van besmettelijke ziekten vergroot (met langlevendheid als uiteindelijk resultaat), maar dat vrouwen met dit profiel waarschijnlijk minder succesvolle zwangerschappen hebben (progentiteit). De selectie voor vruchtbaarheid (een cytokine profiel dat Th-2 type T cellen ondersteunt) is door evolutie geoptimaliseerd door de keuze voor overleving (een cytokine profiel dat Th-1 type T cellen ondersteunt). De

waarschijnlijkheid van een normale vruchtbaarheid nam met meer dan een factor tien toe indien het aangeboren cytokine profiel werd gekarakteriseerd door hoge IL-1 (Th-2) en lage TNFα (Th-1) reactiviteit, in vergelijking met vrouwen die meerdere miskramen hadden ervaren.

In **Hoofdstuk 3** werden verschillende IL-10 promotor polymorfismen (Th-1) op de positie -2849, -1082 en –592 geanalyseerd, met betrekking tot vruchtbaarheid en fecunditeit bij mannelijke en vrouwelijke personen (1116 in totaal), die deelnamen in de Leiden 85-plus studie. Het -2849 A-allel leek in verband te staan met een verminderde IL-10 reactiviteit. Het bleek dat vruchtbaarheid verminderde in relatie met het -2849 A-allel bij vrouwen, ten opzichte van de vrouwelijke G-allel dragers (OR: 2.2, 95% CI: 1.2-4.2). Fecunditeit verminderde in relatie met het -2849 A-allel bij vrouwen in vergelijking tot vrouwelijke G-allel dragers (OR: 0.2, 95% CI: 0,04-0,7). Dit duidt erop dat het IL-10 -2849 AA genotype in verband staat met een verminderde vruchtbaarheid en fecunditeit bij vrouwen, mogelijk als het gevolg van een lager verwachte IL-10 reactiviteit. Bij mannen kon een dergelijk verband niet worden aangetoond.

Wanneer het immuunsysteem het ‘eigen’ weefsel per vergissing aanziet voor ‘niet-eigen’ weefsel en daardoor een ongepaste aanval inzet, dan kan dat een auto-immuunziekte tot gevolg hebben. Reumatoïde artritis (RA) is een voorbeeld van een auto-immuunziekte die voornamelijk voorkomt bij vrouwen. RA is een Th-1 gemedieërde ziekte waarbij cytokines belangrijke mediatoren zijn. Deze cytokines spelen ook een cruciale rol bij de acceptatie- en in stand houden van zwangerschappen.

In **Hoofdstuk 4** wordt onderzocht of de voortplantingsgeschiedenis vóórdat de ziekte begint voorspellend is voor de ernst van RA bij vrouwen. Een cohort van 113 vrouwelijke patiënten werd geïncludeerd uit de polikliniek voor nieuwe reuma patiënten, de early arthritis clinic (EAC), voor de behandeling van de recent geconstateerde RA. Een gestructureerd interview werd afgenomen over hun voortplantingsgeschiedenis. De schade aan de gewrichten werd gemeten door opeenvolgende Röntgen onderzoeken van handen en voeten met gebruikmaking van de aangepaste ‘Sharp score’. De gevonden fecunditeit in dit cohort vrouwen was vergelijkbaar met die van de gegevens van de bevolking als geheel. Er was een even lange tijd tot de uiteindelijke zwangerschap als te verwachten in de normale populatie. Ook had de

verschillende fecunditeit geen invloed op de schade aan de gewrichten naar verloop van tijd. Het aantal miskramen per aantal zwangerschappen bij deze RA patiënten bedroeg 15% per zwangerschap, eveneens vergelijkbaar met die bij de bevolking als geheel (12-15%). Echter, een aanzienlijke toename van schade aan gewrichten werd twee jaar later aangetroffen bij RA patiënten die ten minste één miskraam hadden meegemaakt, dit in vergelijking tot patiënten die nooit een miskraam hadden doorgemaakt (gemiddelde aangepaste Sharp score na twee jaar was 24 (95% CI:15-32) bij de vrouwen die een miskraam hadden doorgemaakt in vergelijking tot een Sharp score van 16 (95% CI: 10-23) bij vrouwen die geen miskramen hadden doorgemaakt. Bij inclusie waren de Sharp scores in beide subgroepen vergelijkbaar. Deze resultaten zouden kunnen aangeven dat het fenotype van gewrichtsvernietiging te maken heeft met het fenotype van gerapporteerde miskramen. wat vergelijkbare genetische risicofactoren voor elk van deze twee karakteristieken suggereert, mogelijk op basis van het aangeboren Th-1/Th-2 fenotype.

Hoofdstuk 5 bespreekt een factor van de stollingscascade in relatie tot fertiliteit en fecunditeit. Factor V Leiden (FVL) is een puntmutatie in het factor V gen (Arg506Gln), dat voorkomt bij 3-10% van het Kaukasische ras. Een nadeel van het dragerschap van het FVL gen is een zevenvoudige toename in het voorkomen van diepe veneuze trombose (DVT). Zwangerschap is in het algemeen een hypercoagulabele status. Er zijn echter ook positieve eigenschappen van FVL aangevoerd. Een verbeterde implantatie kans bij intra-cytoplasmatic sperm injection (ICSI) zwangerschappen is eerder gerapporteerd als de moeder en/of het kind de FVL mutatie droeg. De FVL mutatie zou hierdoor mogelijk de embryo-implantatie ondersteunen. Om dit te onderzoeken werd de voortplantingsgeschiedenis nagevraagd bij 115 vrouwelijke veneuze trombose (VT) patiënten mét de FVL mutatie en 230 vrouwelijke VT patiënten van gelijke leeftijd zónder FVL. De uitkomst van deze studie was dat het dragen van FVL geen invloed heeft op fecunditeit. Miskramen in het eerste trimester bleken minder vaak voor te komen bij dragers van FVL (46%) dan bij niet-dragers (95%) (RR 0.5, 95% CI: 0.3-0.9). Het totaal aantal miskramen bleek echter niet beïnvloed door de FVL status en was gelijk in beide groepen. Deze resultaten suggereren mogelijk dat FVL een beschermend effect heeft gedurende een beginnende zwangerschap en dat een miskraam van embryo's met slechte levensvatbaarheid bij FVL dragers wordt uitgesteld tot het tweede trimester.

Mogelijkerwijs vergroot een grotere locale trombotische activiteit de waarschijnlijkheid van de implantatie van een blastocyste (embryo).

Het bovenstaande in gedachten werd een historisch cohort van 1029 getrouwde mannen en vrouwen geanalyseerd, geboren tussen 1883 en 1914, die meededen aan de Leiden 85-plus studie. In **Hoofdstuk 6** wordt hierover verslag gedaan. Het bevolkingsregister verschafte de data van geboorte, trouwen en geboorte(n) van eventuele kinderen. Er was geen informatie beschikbaar over miskramen. De uitkomst was dat er bij vrouwen geen verband tussen vruchtbaarheid, fecunditeit en FVL status werd aangetoond. Bij mannen werd een onverwacht, maar statistisch zeer significant hogere fecunditeit (kortere periode tussen trouwen en eerstgeborene) bij FVL dragers gevonden in vergelijking tot niet-dragers (RR: 3.5; 95% CI: 2.1-5.7). Er was geen verband te vinden tussen de FVL mutatie en fertiliteit (wel of geen kinderen) of de familiegrootte bij zowel mannen als vrouwen. Over de mogelijke verklaringen van deze bevinding kunnen we slechts speculatief zijn. FVL zou de mannelijke vruchtbaarheid kunnen bevorderen, dus dat het FVL gen mogelijk verbonden is aan een vruchtbaarheidsgen en dat hierdoor de hoeveelheid of beweeglijkheid van het sperma toeneemt. Of, meer waarschijnlijk, dat de aanwezigheid van een FVL mutatie in een embryo de implantatiekans in een FVL negatieve moeder kan vergroten.

In **Hoofdstuk 7** worden de resultaten van de alle onderzoeken samengevat en besproken in breder perspectief.

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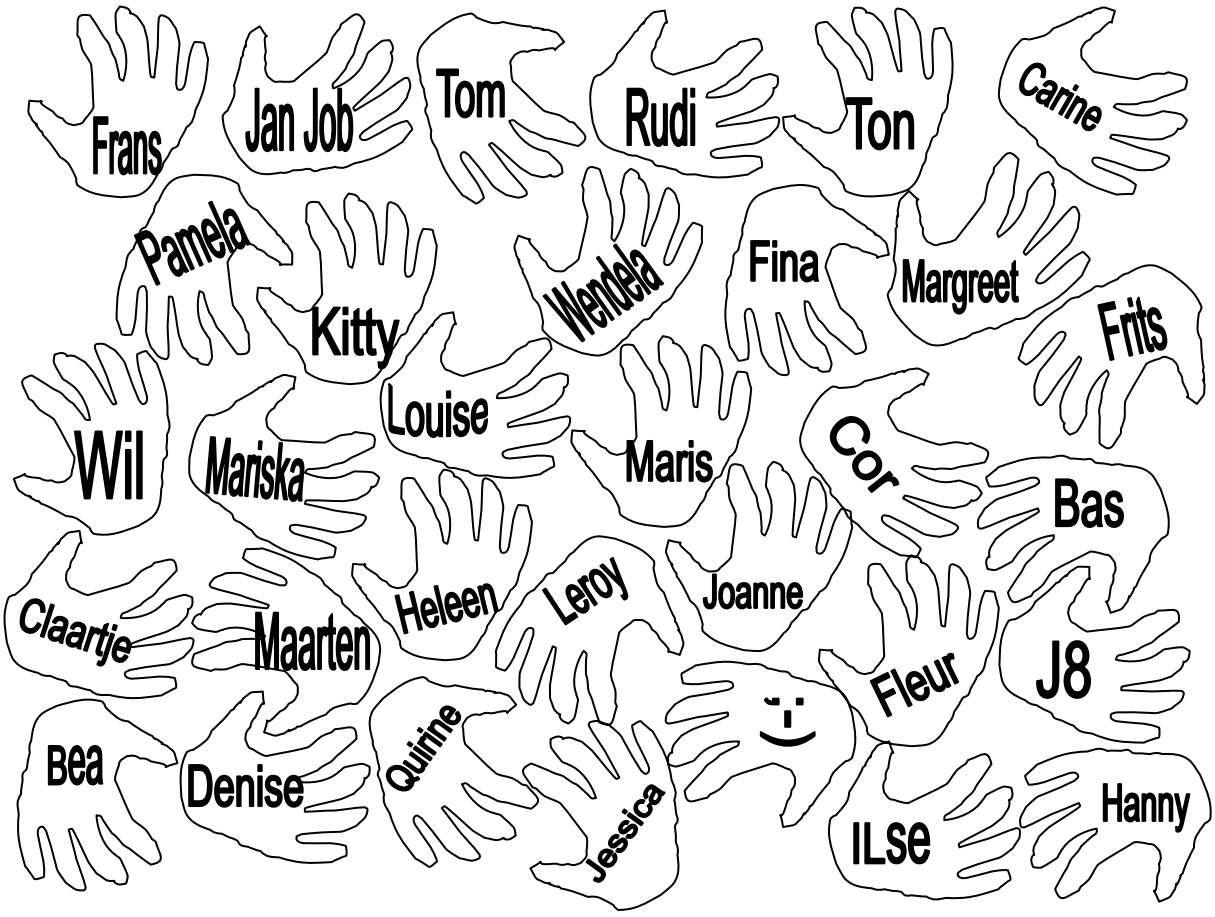

THANKS!

CURRICULUM VITAE

Frédérique Margo van Dunné werd geboren op 30 augustus 1968 in Dampier, Australië waar haar vader was uitgezonden voor zijn werk. De lagere school heeft zij achtereenvolgens in Engeland, Saoedi-Arabië en Nederland doorlopen. Het VWO heeft zij aan het Huygens Lyceum te Voorburg voltooid. Van 1986 tot 1992 studeerde zij medicijnen aan de Rijksuniversiteit Leiden. Tijdens haar studie interesseerde Prof. Dr. M.J.N.C. Keirse haar voor het eerst in wetenschappelijk onderzoek. Deze interesse werd versterkt tijdens haar vijf maanden durend keuze co-schap in het 'perinatal lab' van het Monash Medical Center in Melbourne, Australië, onder leiding van Prof. S. P. Brennecke. Na het afronden van haar co-schappen in 1994 werkte zij ruim een jaar als AGNIO op de afdeling gynaecologie en obstetrie van het Reinier de Graaf Gasthuis te Delft. In 1996 begon zij haar opleiding tot gynaecoloog, eerst in het Ziekenhuis Leyenburg te Den Haag (opleiders Dr. J.P. Holm en dr. P.A. de Jong) en vervolgens in het Leids Universitair Medisch Centrum (opleider Prof. Dr. H.H.H. Kanhai). Tijdens haar opleiding begon ze in 2000 op de afdeling verloskunde van het LUMC met onderzoek, in samenwerking met de afdeling reumatologie. Deze samenwerking breidde zich al snel uit met de afdeling gerontologie. Nadat zij in 2002 haar opleiding tot gynaecoloog afronde zette deze samenwerking zich voort en leidde uiteindelijk tot dit proefschrift. Van 2003 tot 2005 werkte zij als fellow Maternal Fetal Medicine in het Royal Women's Hospital in Melbourne, Australië. Vanaf mei 2004 is zij tevens ingeschreven als gynaecoloog in Australië (Fellow of the Royal Australian and New Zealand College of Obstetrics and Gynaecology, FRANZCOG). Sinds 1 januari 2006 is zij als staflid werkzaam bij de afdeling verloskunde en prenatale diagnostiek in het Erasmus Medisch Centrum te Rotterdam. Frédérique is getrouwd met Jan Job de Vries Robbé en samen zijn zij de trotse ouders van de tweeling Pieter en Mathijs en dochter Noortje.

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Frédérique van Dunné was born in Dampier, Western Australia on the 30th of August 1968. Her father was stationed there for the dredging of the iron ore harbour. She attended primary school in England, Saudi Arabia and The Netherlands, and secondary school at the Huygens Lyceum in Voorburg, The Netherlands. She commenced Medical School at the Rijksuniversiteit Leiden in 1986. During her studies Prof. M. Keirse initiated her in medical research. This interest furthered during a 5 month research project at the perinatal lab at Monash Medical Center in Melbourne, Australia (under the supervision of Prof. S.P. Brennecke). After the completion of her internships in 1994 she worked as a resident at the department of Obstetrics and Gynaecology at the Reinier de Graaf Gasthuis in Delft, The Netherlands. She commenced her training as an O&G registrar in 1996, first at the Ziekenhuis Leyenburg in The Hague (supervisors dr. J.P. Holm and dr. P.A. de Jong) and then at the Leids Universitair Medisch Centrum (LUMC) in Leiden, The Netherlands (supervisor Prof. Dr. H.H.H. Kanhai). During her O&G training she commenced her research in collaboration with the department of Rheumatology at the LUMC. The partnership subsequently extended to the department of Gerontology at the LUMC which resulted in this thesis. From 2003 to 2005 she worked as a fellow Maternal Fetal Medicine at the Royal Women's Hospital in Melbourne, Australia. In May 2004 she was accepted as a Fellow of the Royal Australian and New Zealand College of Obstetrics and Gynaecology, (FRANZCOG). She now works as a Consultant at the department of Obstetrics and Prenatal Diagnosis at the Erasmus Medical Center in Rotterdam, The Netherlands. Frédérique is married to Jan Job de Vries Robbé and together they are the proud parents of twins Pieter and Mathijs and daughter Elenora.