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

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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.

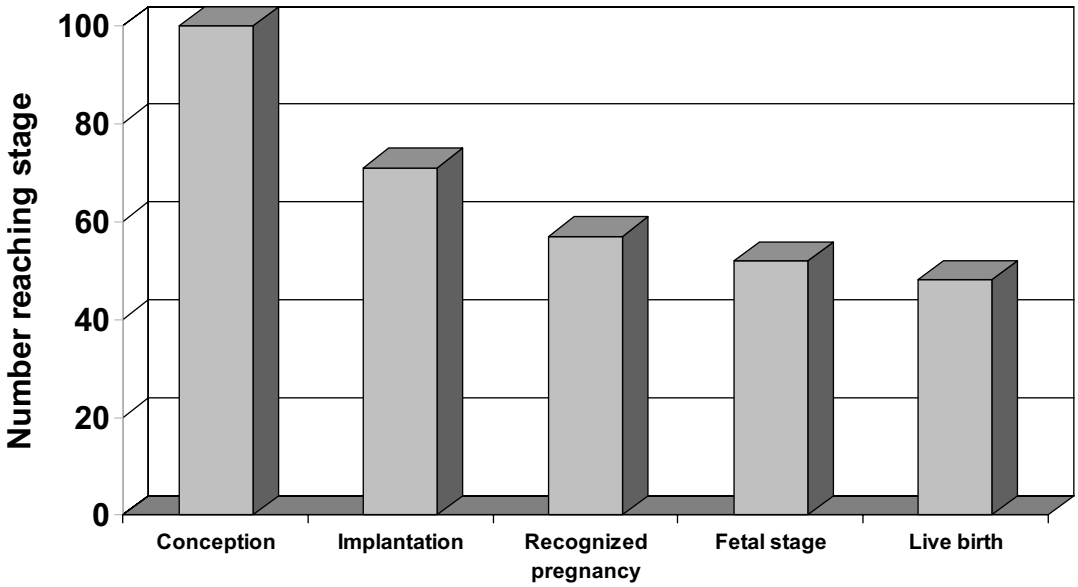

Figure 1. The fate of a fertilised ovum is a poor one[18].

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].

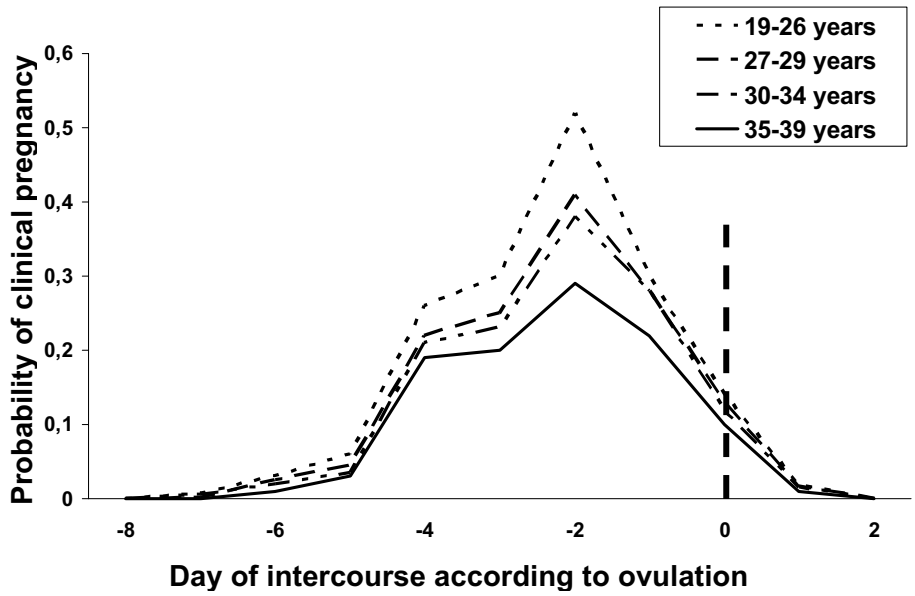

Figure 2. Probability of clinical pregnancy following intercourse on a given day relative to ovulation (day 0) for women of average fertility aged 19–26, 27–29, 30–34 and 35–39 years (European Study of Daily Fecundability, 433 pregnancies), adjusted for male partner's age[19].

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].

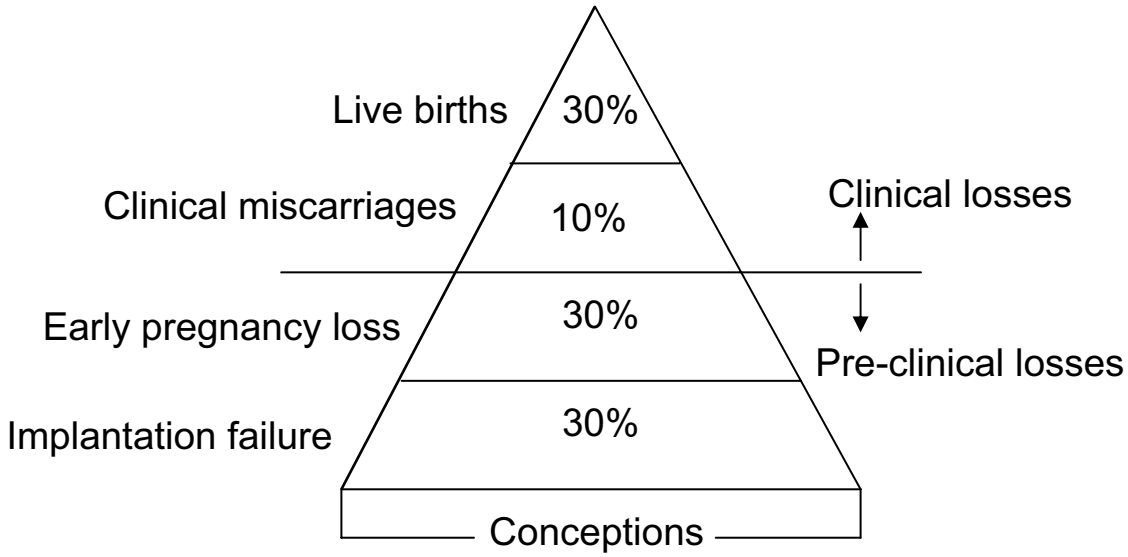

Figure 3. The pregnancy loss Iceberg; an overview of the outcome of spontaneous human pregnancy. A total of 70% of conceptions are lost prior to live birth. The majority of these losses are prior to the time of the missed menstruation and are not noticed. Adapted from Macklon 2002[15].

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.

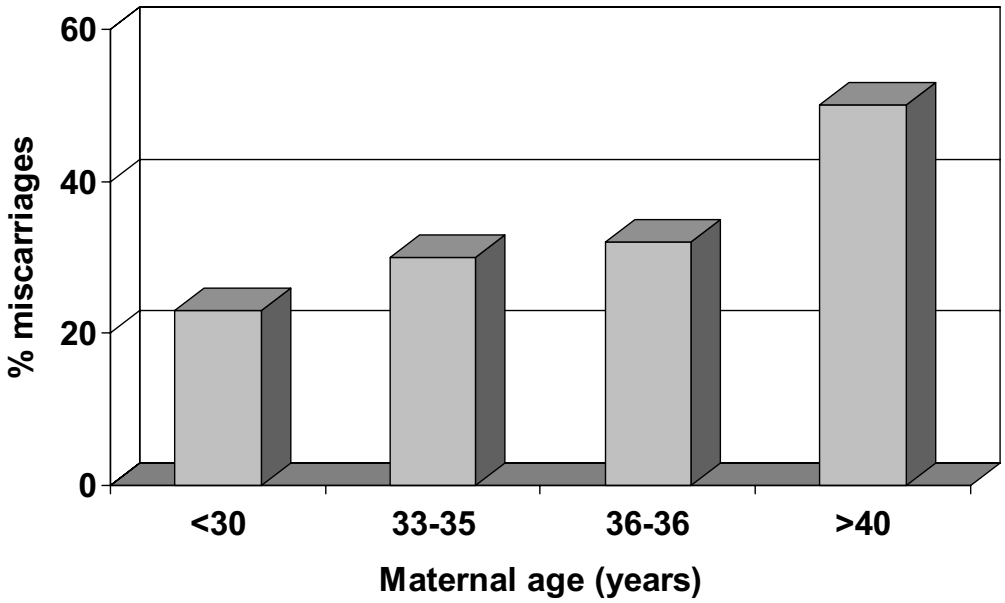

Figure 4. Influence of maternal age on outcome of subsequent pregnancy. After Clifford 1997[39].

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.

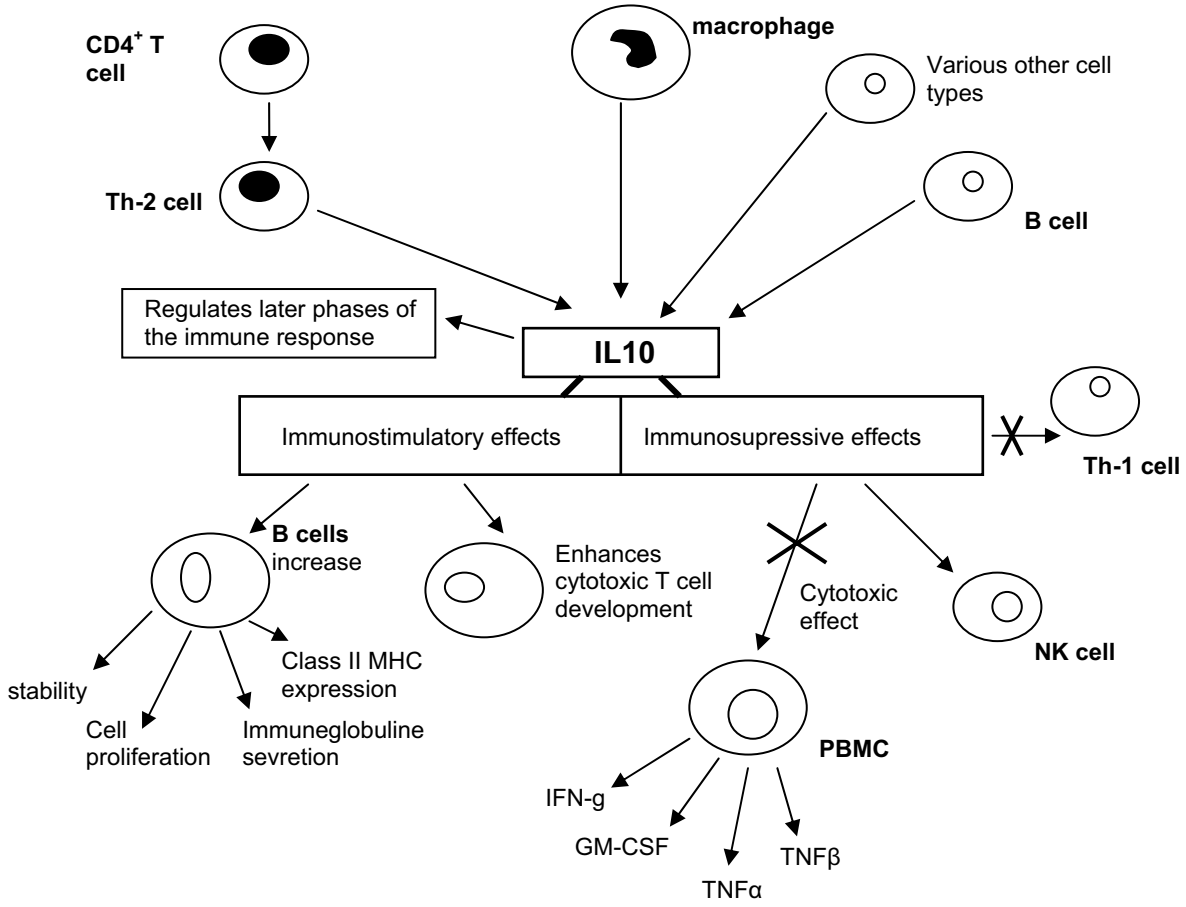

Figure 5. A simplified schematic figure of the IL-10 mechanism. Activation of various cells produce IL-10, IL-10 then plays an important immunostimulatory as well as inhibitory role. The inhibitory effect is indicated by 'X'.After Conti et al, 2003[59].

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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