Unexplained recurrent miscarriage

Kaandorp, S.P.

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Introduction and outline of the thesis

Recurrent miscarriage is a traumatic event, which may lead to symptoms of depression, anxiety, lowered self-esteem and other psychosocial consequences.[1] One percent of all women trying to conceive will have three miscarriages or more in their lives.[2] If recurrent miscarriage is defined as two or more miscarriages, the proportion of women with recurrent miscarriage increases to 5%.

Established causes for recurrent miscarriage are structural chromosome abnormalities of one of the partners, uterine abnormalities, elevated random level of homocysteine and antiphospholipid syndrome.[3, 4] In half of the women with recurrent miscarriage no apparent cause can be found.[5, 6] Cytogenetic abnormalities in miscarriage tissue occur in around 50% of women with a sporadic miscarriage as well as in women with recurrent miscarriage.[7-14] Hence in 25% of the women with two preceding miscarriages or in 12 % of women with three preceding miscarriages, cytogenetic abnormalities can explain their miscarriages. Since cytogenetic analysis of miscarriage tissue is not standard care, this group is not identified and thus included in the group of women with unexplained recurrent miscarriage.

This thesis focuses on the clinical entity which is referred to as *unexplained* recurrent miscarriage. It explores existing knowledge and investigates the effect of anticoagulant interventions.

One of the pathophysiological pathways of interest is based on the observed association between recurrent miscarriage and thrombophilia. This concerns both acquired thrombophilia as in antiphospholipid syndrome, as well as inherited thrombophilia, such as deficiencies of the natural anticoagulants antithrombin, protein C or protein S, and the gain of function mutations factor V Leiden and prothrombin 20210A. In large family cohort studies, women with inherited thrombophilia had a slightly higher risk for recurrent miscarriage than their relatives without thrombophilia.[15-17] Systematic reviews of population based case control studies endorse these findings.[18-21]

It was initially hypothesized that hypercoagulability with subsequent thrombosis of placental vasculature would be a pathophysiological mechanism.[22] Later, a non-thrombotic theory was proposed based on a study showing that antiphospholipid antibodies inhibit normal trophoblast invasion.[23] These data were backed up by in vitro experiments showing that antiphospholipid antibodies inhibit extravillous trophoblast differentiation. Failure of trophoblast differentiation and subsequent placentation was thus suggested to be an underlying pathophysiological mechanism of pregnancy loss in antiphospholipid syndrome.[24] In addition to these human data, tissue factor initiated activation of the blood coagulation at the feto-maternal interface causing early pregnancy loss was convincingly demonstrated in thrombomodulin-deficient mice. These mice, which lack the natural anticoagulant protein C pathway, were unable to carry their fetuses beyond 8.5 days post coitum. Activated coagulation factors were found that

induced cell death and inhibited growth of throphoblast cells. Administration of heparin or aspirin to the mice delayed this process but could not prevent early pregnancy loss.[25]
Despite the lack of understanding of the underlying pathophysiology in the majority of women with unexplained recurrent miscarriage, several interventions have been suggested to improve the chance of a live birth. Some interventions are based on presumed abnormalities in the immune and coagulation system and have been introduced without adequate clinical data to support a positive effect. For example, although allo-immunity, such as incompatibility of human leukocyte antigens in couples, has not been firmly established as an underlying pathophysiological mechanism in humans, several immunologic interventions such as paternal cell immunization and immunoglobulin infusions have been advocated, and none have shown a significant beneficial effect on the chances of subsequent live birth.[26]
The same is true for anticoagulant interventions. Treatment with aspirin and heparin has been suggested to improve live birth rate in women with antiphospholipid syndrome and recurrent miscarriage, although pathofysiological underpinning is meagre and evidence from adequate trials is non conclusive.[27] Some clinicians tend to extrapolate this presumed beneficial effect to women with unexplained recurrent miscarriage. Hypercoagulability was coined as a cause for unexplained recurrent miscarriage because thrombosis of placental vessels and infarctions were found in some cases.[28] Non-controlled studies showed a beneficial effect on live birth of anticoagulants in women with inherited thrombophilia and adverse obstetric events, including recurrent miscarriage, in their history,[29-34] but adequately performed and controlled trials on the efficacy of interventions directed at hypercoagulability such as aspirin or heparin, are too scarce to recommend their use in women with unexplained recurrent miscarriage.[35] Quasi randomised controlled trials reported contradicting results.[36-39]
Since unexplained recurrent miscarriage is a stressful condition for the couple, most women are eager and willing to try any form of treatment. Nevertheless, clinicians should never resort to quackery. It is their moral and professional obligation to withhold non evidence based treatment and to initiate large scale collaboration in evaluation research in order to achieve evidence and improvement in guidelines.

Background of the research described in this thesis

In 2005, when we started the studies that led to this thesis, in at least 50% of the women with recurrent miscarriage, the condition remained unexplained. We aimed to identify potential pathophysiological mechanisms that could lead to targeted interventions.
First, we performed a review of the literature on the association between inherited thrombophilia and pregnancy associated complications, including recurrent miscarriage,

and venous thromboembolism. The focus of the review was the relevance of thrombophilia testing for clinical management.

Apart from women with thrombophilia also women with Philadelphia chromosome negative myeloproliferative disorders (MPD) have an increased risk of experiencing miscarriages compared with the general population.[40] Recently, a somatic gain-of-function mutation associated with MPD was found.[41] Contradicting results about a possible association of this mutation with recurrent miscarriage are reported. In view of these discordant data we aimed to investigate the prevalence of JAK2V617F mutation in a well described cohort of women with unexplained recurrent miscarriage.

It has been claimed that conventional cytogenetic analysis of miscarriage tissue in the second and subsequent miscarriages or all miscarriages in women with advanced maternal age is informative for both clinician and patient in the evaluation of recurrent miscarriage.[42, 43] Theoretically, if in all miscarriage samples of women with unexplained recurrent miscarriage an explanatory cytogenetic abnormality could be identified the diagnosis 'unexplained' recurrent miscarriage would no longer be valid. As the debates on the usefulness of analysing miscarriage tissue go on, a relatively new molecular technique, multiplex ligation-dependent probe amplification (MLPA), has been introduced. This technique can be used to investigate miscarriage tissue. We aimed to compare the success rate of telomere-MLPA with conventional karyotyping and to explore the potential ability of telomere-MLPA to determine small subtelomeric cytogenetic abnormalities that could not be detected with conventional karyotyping.

Aspirin and low-molecular-weight heparin were frequently prescribed for women with unexplained recurrent miscarriage or those with recurrent miscarriage and inherited thrombophilia.[44, 45] We performed a review on the efficacy and safety of anticoagulant treatments for women with unexplained recurrent miscarriage, identifying a lack of evidence coinciding with an increased tendency to prescribe these drugs. We then performed a randomised controlled trial to investigate whether aspirin combined with low-molecular-weight heparin or aspirin alone, as compared with placebo, would improve the live birth rate among women with unexplained recurrent miscarriage.

Since expectant management remains the treatment of choice in many couples with unexplained recurrent miscarriage, knowledge on time to natural conception is very important. We aimed to assess time to natural conception in a cohort of women with unexplained recurrent miscarriage.

Outline of the thesis

Chapter 2 describes the current forms of inherited thrombophilia and their underlying pathophysiology and epidemiology. The association of inherited thrombophilias and

pregnancy-associated complications like recurrent miscarriage and possible clinical implications are reported.

Chapter 3 reports on the role of JAK2V617F mutation in women with unexplained recurrent miscarriage. We assessed the prevalence of JAK2V617F mutation in a well described cohort of 147 women with unexplained recurrent miscarriage.

Chapter 4 describes two genetic techniques performed in miscarriage tissue from women with unexplained recurrent miscarriage. Telomere multiplex ligation-dependent probe amplification and conventional karyotyping was performed on 29 samples of miscarriage tissue. The success rates and results of both genetic techniques were compared.

Chapter 5 provides a Cochrane systematic review to evaluate the efficacy and safety of anticoagulant agents, such as aspirin and heparin in women with unexplained recurrent miscarriage. Two studies were included in the review, the results are presented.

Chapter 6 reports the results of a multicenter randomised placebo-controlled trial performed: the Anticoagulants for Living Fetuses (ALIFE) study. We investigated whether aspirin combined with low-molecular-weight heparin or aspirin alone, as compared with placebo, would improve the live-birth rate among 364 women with unexplained recurrent miscarriage.

Chapter 7 evaluates time to natural conception in a prospective nested cohort study (ALIFE study). 251 women not pregnant at the moment that unexplained recurrent miscarriage was diagnosed were included and time to natural conception was calculated.

Chapter 8 presents the summary of this thesis and provides suggestions for future research.

References

1. Serrano F, Lima ML. Recurrent miscarriage: psychological and relational consequences for couples. Psychol Psychother 2006;79(Pt 4):585-594.
2. Rai R, Regan L. Recurrent miscarriage. Lancet 2006;368(9535):601-611.
3. Christiansen OB, Nybo Andersen AM, Bosch E et al. Evidence-based investigations and treatments of recurrent pregnancy loss. Fertil Steril 2005;83(4):821-839.
4. Jauniaux E, Farquharson RG, Christiansen OB, Exalto N. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. Hum Reprod 2006;21(9):2216-2222.
5. Crosignani PG. Genetic aspects of female reproduction. Human Reproduction Update 2008;1-15.
6. Regan L, Rai R. Epidemiology and the medical causes of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14(5):839-854.
7. Carp H, Toder V, Aviram A, Daniely M, Mashiach S, Barkai G. Karyotype of the abortus in recurrent miscarriage. Fertil Steril 2001;75(4):678-682.
8. Goddijn M, Leschot NJ. Genetic aspects of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14(5):855-865.
9. Marquard K, Westphal LM, Milki AA, Lathi RB. Etiology of recurrent pregnancy loss in women over the age of 35 years. Fertil Steril 2010;94(4):1473-1477.
10. Ogasawara M, Aoki K, Okada S, Suzumori K. Embryonic karyotype of abortuses in relation to the number of previous miscarriages. Fertil Steril 2000;73(2):300-304.
11. Simpson JL, Bombard A. Chromosomal abnormalities in spontaneous abortion: frequency, pathology and genetic counselling. In: Bennett M.J., Edmonds D.K., editors. Spontaneous and recurrent abortion. Oxford: Blackwell Scientific Publications; 1987:51-76.
12. Stephenson MD, Awartani KA, Robinson WP. Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. Hum Reprod 2002;17(2):446-451.
13. Stern JJ, Dorfmann AD, Gutierrez-Najar AJ, Cerrillo M, Coulam CB. Frequency of abnormal karyotypes among abortuses from women with and without a history of recurrent spontaneous abortion. Fertil Steril 1996;65(2):250-253.
14. Sullivan AE, Silver RM, LaCoursiere DY, Porter TF, Branch DW. Recurrent fetal aneuploidy and recurrent miscarriage. Obstet Gynecol 2004;104(4):784-788.
15. Preston FE, Rosendaal FR, Walker ID et al. Increased fetal loss in women with heritable thrombophilia. Lancet 1996;348(9032):913-916.
16. Sanson BJ, Friederich PW, Simioni P et al. The risk of abortion and stillbirth in antithrombin-, protein C-, and protein S-deficient women. Thromb Haemost 1996;75(3):387-388.
17. Meinardi JR, Middeldorp S, de Kam PJ et al. Increased risk for fetal loss in carriers of the factor V Leiden mutation. Ann Intern Med 1999;130(9):736-739.
18. Alfirevic Z, Roberts D, Martlew V. How strong is the association between maternal thrombophilia and adverse pregnancy outcome? A systematic review. Eur J Obstet Gynecol Reprod Biol 2002;101(1):6-14.
19. Dudding TE, Attia J. The association between adverse pregnancy outcomes and maternal factor V Leiden genotype: a meta-analysis. Thromb Haemost 2004;91(4):700-711.
20. Kovalevsky G, Gracia CR, Berlin JA, Sammel MD, Barnhart KT. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: a meta-analysis. Arch Intern Med 2004;164(5):558-563.
21. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet 2003;361(9361):901-908.

22. Dizon-Townson DS, Meline L, Nelson LM, Varner M, Ward K. Fetal carriers of the factor V Leiden mutation are prone to miscarriage and placental infarction. Am J Obstet Gynecol 1997;177(2):402-405.

23. Sebire NJ, Regan L, Rai R. Biology and pathology of the placenta in relation to antiphospholipid antibody-associated pregnancy failure. Lupus 2002;11(10):641-643.

24. Quenby S, Mountfield S, Cartwright JE, Whitley GS, Chamley L, Vince G. Antiphospholipid antibodies prevent extravillous trophoblast differentiation. Fertil Steril 2005;83(3):691-698.

25. Isermann B, Sood R, Pawlinski R et al. The thrombomodulin-protein C system is essential for the maintenance of pregnancy. Nat Med 2003;9(3):331-337.

26. Porter TF, LaCoursiere Y, Scott JR. Immunotherapy for recurrent miscarriage. Cochrane Database Syst Rev 2006;(2):CD000112.

27. Empson M, Lassere M, Craig J, Scott J. Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. Cochrane Database Syst Rev 2005;(2):CD002859.

28. Rushton DI. Placental pathology in spontaneous miscarriage. In: Beard RW, Sharp F, editors. Early pregnancy loss: mechanisms and treatment. London: RCOG; 1988:149-158.

29. Kupferminc MJ, Fait G, Many A et al. Low-molecular-weight heparin for the prevention of obstetric complications in women with thrombophilias. Hypertens Pregnancy 2001;20(1):35-44.

30. Riyazi N, Leeda M, de Vries JI, Huijgens PC, van Geijn HP, Dekker GA. Low-molecular-weight heparin combined with aspirin in pregnant women with thrombophilia and a history of preeclampsia or fetal growth restriction: a preliminary study. Eur J Obstet Gynecol Reprod Biol 1998;80(1):49-54.

31. Grandone E, Brancaccio V, Colaizzo D et al. Preventing adverse obstetric outcomes in women with genetic thrombophilia. Fertil Steril 2002;78(2):371-375.

32. Brenner B, Hoffman R, Blumenfeld Z, Weiner Z, Younis JS. Gestational outcome in thrombophilic women with recurrent pregnancy loss treated by enoxaparin. Thromb Haemost 2000;83(5):693-697.

33. Brenner B, Hoffman R, Carp H, Dulitsky M, Younis J. Efficacy and safety of two doses of enoxaparin in women with thrombophilia and recurrent pregnancy loss: the LIVE-ENOX study. J Thromb Haemost 2005;3(2):227-229.

34. Carp H, Dolitzky M, Inbal A. Thromboprophylaxis improves the live birth rate in women with consecutive recurrent miscarriages and hereditary thrombophilia. J Thromb Haemost 2003;1(3):433-438.

35. Di Nisio M, Peters L, Middeldorp S. Anticoagulants for the treatment of recurrent pregnancy loss in women without antiphospholipid syndrome. Cochrane Database Syst Rev 2005;(2):CD004734.

36. Badawy AM, Khiary M, Sherif LS, Hassan M, Ragab A, Abdelall I. Low-molecular weight heparin in patients with recurrent early miscarriages of unknown aetiology. J Obstet Gynaecol 2008;28(3):280-284.

37. Dolitzky M, Inbal A, Segal Y, Weiss A, Brenner B, Carp H. A randomized study of thromboprophylaxis in women with unexplained consecutive recurrent miscarriages. Fertil Steril 2006;86(2):362-366.

38. Fawzy M, Shokeir T, El-Tatongy M, Warda O, El-Refaiey AA, Mosbah A. Treatment options and pregnancy outcome in women with idiopathic recurrent miscarriage: a randomized placebo-controlled study. Arch Gynecol Obstet 2008;278(1):33-38.

39. Tulppala M, Marttunen M, Soderstrom-Anttila V et al. Low-dose aspirin in prevention of miscarriage in women with unexplained or autoimmune related recurrent miscarriage: effect on prostacyclin and thromboxane A2 production. Hum Reprod 1997;12(7):1567-1572.

40. Passamonti F, Randi ML, Rumi E et al. Increased risk of pregnancy complications in patients with essential thrombocythemia carrying the JAK2 (617V>F) mutation. Blood 2007;110(2):485-489.

41. Baxter EJ, Scott LM, Campbell PJ et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365(9464):1054-1061.

42. Carp HJ. Recurrent miscarriage: genetic factors and assessment of the embryo. Isr Med Assoc J 2008;10(3):229-231.

43. Stephenson M, Kutteh W. Evaluation and management of recurrent early pregnancy loss. Clin Obstet Gynecol 2007;50(1):132-145.

44. Goddijn M, van d, V, Ankum WM, Bonsel GJ, Leschot NJ, Boer K. [No consensus on the definition, diagnosis and treatment of habitual abortion in the Netherlands]. Ned Tijdschr Geneeskd 1999;143(17):897-902.

45. Franssen MT, Korevaar JC, van d, V, Boer K, Leschot NJ, Goddijn M. Management of recurrent miscarriage: evaluating the impact of a guideline. Hum Reprod 2007;22(5):1298-1303.