Chromosome abnormalities in first-trimester pregnancy loss

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General introduction
Introduction

In human reproduction, pregnancy loss is a frequent event. Only approximately 30% of all conceptions progress to a successful delivery\(^1\). Early pregnancy loss, at present most often regarded as subclinical or occult pregnancy loss, ends before pregnancy is clinically detected. It occurs in about 13-22% of all pregnancies detected by hCG testing\(^2\,^3\).

First-trimester pregnancy loss, consists of sporadic miscarriage, ectopic pregnancy, recurrent miscarriage and gestational trophoblastic disease\(^4\). Sporadic miscarriages account for most first-trimester pregnancy losses, its incidence accounting for 12-15% of all clinically recognized pregnancies\(^5\,^6\). The vast majority of sporadic miscarriages occur before 12 weeks of gestation\(^7\). In sporadic miscarriage, a lot of data concerning the frequency and type of chromosome abnormalities is available\(^8\,^17\). In ectopic pregnancies however, less is known about the role of chromosome abnormalities. In recurrent miscarriage, insufficient light has, as yet, been shed on the consequences of parental chromosome abnormalities.

This thesis deals with chromosome abnormalities in first-trimester pregnancy loss, specifically ectopic pregnancies and recurrent miscarriage.

Ectopic pregnancy

Ectopic pregnancy is defined as an implantation occurring outside the uterine cavity\(^18\). Up to 95% of ectopic gestations are located in the Fallopian tube, other implantation sites being the ovary, cervix, abdominal cavity, and occasionally liver, spleen and vagina\(^19\).

Ectopic pregnancies constitute approximately 1-2% of all clinically recognized pregnancies\(^20\). Several risk factors and associated conditions for the development of ectopic pregnancies have been found. Among these, the strongest risk factors are: previous ectopic pregnancy, previous tubal surgery, documented tubal pathology, and in utero DES exposure\(^21,22\).

Previous genital infections, infertility, current IUD use, and more than one sexual partner are associated with a mildly increased risk. Previous pelvic and/or abdominal surgery, smoking and an early age at first sexual intercourse are associated with a slightly increased risk\(^21,22\).

The etiology of about 30% of cases still remains unknown\(^23\). Suggested factors are under-reporting of pelvic inflammatory disease, altered transport mechanisms by an oestrogen/progesteron imbalance, and fetal chromosome abnormalities\(^18,24,25\).

So far, studies focusing on chromosome abnormalities in ectopic pregnancies, have reported
a wide range of prevalence of abnormalities\textsuperscript{25-35}. Because of these inconclusive findings, further investigation seems justified.

**Recurrent miscarriage**

Recurrent miscarriage is ill-defined, probably because of the uncertainty, complexity and heterogenety surrounding the condition\textsuperscript{36}. When defined as three or more miscarriages, irrespective of parity, recurrent miscarriage is estimated to occur in 0.5-1\% of all couples trying to conceive\textsuperscript{37}. When women with two or more pregnancy losses are included, the scale of the problem increases to include more than 3\% of the couples\textsuperscript{6}.

Recent evidence shows that genetic abnormalities of the conceptions account for the majority of cases with recurrent miscarriage\textsuperscript{38}. If only one miscarriage occurs, it is usually regarded as a sporadic miscarriage by chance. In sporadic miscarriages the frequency of numerical chromosome abnormalities of the conceptus is high, approximately 50-60\%.

In a subgroup of couples with recurrent miscarriage, the cause of the miscarriages can be a repetition of numerical chromosome abnormalities\textsuperscript{39}. It is known that an effect of maternal age on the frequency of trisomies exists\textsuperscript{40,41}. Among women <25 years, about 2\% of all clinically recognized pregnancies are trisomic, but among women aged 40 this value approaches 25\%\textsuperscript{40}. The age-associated increased risk of a miscarriage is largely attributable to abnormalities in the oocyte. Most of the trisomies result from a maternal meiotic error, which preferentially occurs during the first meiotic division\textsuperscript{42}. Proposed mechanisms at the molecular level are meiotic spindle abnormalities\textsuperscript{43}, and single chromatid abnormalities\textsuperscript{44}.

If more recent techniques, like (semi-)direct chromosome technique, fluorescence in situ hybridization (FISH) or comparative genomic hybridization (CGH) are used, the prevalence of chromosome abnormalities in sporadic miscarriage is higher when compared to culture\textsuperscript{45-47}.

When these results are extrapolated and related to couples with recurrent miscarriage, the relative proportion of recurrence of chromosome abnormalities can be supposed to be even higher. In another subgroup of couples with recurrent miscarriage, although less frequent, an important genetic factor accounting for the recurrence of miscarriages exists. Either the woman and/or man carries a structural chromosome abnormality. Carriership of a structural chromosome abnormality is present in 4.7\% of all couples ascertained for two or more miscarriages, and the abnormality is most commonly a balanced reciprocal or Robertsonian translocation\textsuperscript{48}. 
Still, some non-genetic causes may also explain the recurrence of miscarriages. Among them, the best established risk factor for recurrent miscarriage is the presence of antiphospholipid antibodies. Antiphospholipid antibodies – lupus anticoagulant (LA) and anticardiolipin antibodies of the IgG or IgM class – are present in 15% of women with recurrent miscarriages. The live birth rate (live born babies per total number of clinically recognized pregnancies) in these women may be as low as 10%. It is difficult to assess the importance of other non-genetic factors, like uterine structural abnormalities. Amongst women with recurrent miscarriage they seem to play a minor role. Contrary to prior conviction, available evidence rules out that a large part is played by endocrine factors. Well controlled diabetes mellitus and treated thyroid dysfunction are no risk factors for recurrent miscarriage.

Thus far, the main factors predicting the chance of a livebirth in couples with recurrent miscarriage are maternal age, number of previous miscarriages, the presence of antiphospholipid antibodies, and parental carriership of structural chromosome abnormalities.

Possible association between recurrent miscarriage and ectopic pregnancies

A two- to four-fold increase in ectopic pregnancies has been reported in women with recurrent miscarriage. Also, the risk of recurrence of an ectopic pregnancy was reported to be higher in these women (OR = 3.4 to 11.9; 95% CI 1.6-7.3 to 4.3 to 32.6, depending on the number of miscarriages). In another study, a tendency to more miscarriages was found both before and after the ectopic pregnancy. These associations suggest some common risk factors for both reproductive failures. Nevertheless, no clear causal factor has been found so far.

Remarkably, two other phenomena are shared by miscarriages and ectopic pregnancies, i.e. the age related risk and their seasonal variability.

High maternal age is a risk factor for miscarriage and ectopic pregnancy. As stated earlier, an effect of maternal age has been demonstrated on the frequency of trisomies in miscarriages. In ectopic pregnancies, no such studies regarding the origin of the age related risk are known. A higher proportion of chromosome abnormalities could be one explanation, but being exposed more frequently to a pelvic inflammatory disease (PID), especially Chlamydia infection, at higher maternal age could be another explanation. The second common factor in miscarriages, and ectopic pregnancy, not yet explained, is the seasonal variability i.e. an increase in these phenomena in autumn and winter time.
Chromosome abnormalities in ectopic pregnancies and recurrent miscarriage

Before the writing of this thesis, conflicting evidence existed regarding the role of chromosome abnormalities in ectopic pregnancies. Highly varying numbers of chromosome abnormalities were reported, determined by different techniques. For three reasons new studies were needed to determine a more accurate frequency of chromosome abnormalities in well defined ectopic pregnancies.

Firstly, the older studies were performed in another diagnostic era, before non-invasive diagnostic strategies were introduced. By introducing these strategies, the surgical removal of non-vital ectopic pregnancies, destined to disappear spontaneously was no longer necessary. Surgical removal of ectopic pregnancies could then be restricted to vital ectopic pregnancies, in which growth was likely to continue. It is possible that in this subgroup of vital ectopic pregnancies a relatively low number of chromosome abnormalities is present.

Secondly, with the availability of newer molecular biological techniques, like PCR, FISH and CGH, it seemed feasible to obtain a higher number of successful chromosomal diagnoses, when compared to conventional techniques.

Thirdly, data regarding maternal age and gestational age was not available in some older studies. Furthermore, before the start of the present studies, no evidence was available as to whether it was possible to identify chromosome abnormalities in ectopic pregnancies by histological examination.

At this moment in time, there is no consensus on whether carriership analysis is useful in couples with recurrent miscarriage and advanced maternal age. Testing for carriership is time-consuming and therefore expensive. The yield of the test is relatively low, as a structural chromosome abnormality is found only in about 4.7% of couples with recurrent miscarriage. Women with recurrent miscarriage and advanced maternal age are eligible for prenatal diagnosis, in addition to parental karyotyping. The effectiveness of offering both screening programmes - karyotyping for parental carriership and prenatal chromosome studies of the fetus - to this group of women has not yet been determined.

We aim to investigate whether withholding carriership detection in the subgroup with recurrent miscarriage at maternal age of 36 years or higher is justified. Therefore, the frequency of carriership in couples with recurrent miscarriage at maternal age <36 years, and >36 years needs to be determined, as well as possible unbalanced offspring after having established parental carriership.
The aim of the thesis

The aim of this thesis is to address the following questions:

1. What is the prevalence of chromosome abnormalities in ectopic pregnancies?

2. Can multiplex fluorescence polymerase chain reaction (MF-PCR) and/or fluorescence in situ hybridization (FISH) detect chromosome abnormalities in ectopic pregnancies?

3. Are histological features associated with cytogenetic abnormalities in ectopic pregnancies?

4. What is known about the frequency and type of chromosome abnormalities in sporadic miscarriage and recurrent miscarriage?

5. What is the current policy on diagnosis and treatment of recurrent miscarriage in the Netherlands?

6. What is the projected prevalence of parental structural chromosome abnormalities in couples with recurrent miscarriage, especially in the subgroup with maternal age ≥ 36 years?
Outline of the Thesis

Chapter 2
Chorionic villi of 30 ectopic pregnancies were karyotyped by (semi-)direct technique. As controls, ten cases of intrauterine pregnancies were investigated.

Chapter 3
Seventy chorionic villi samples of ectopic pregnancies were studied by multiplex fluorescent polymerase chain reaction (MF-PCR). Fluorescence in situ hybridization (FISH) was performed where results of MF-PCR showed aneuploidy, in case of uninterpretable MF-PCR results, and in ten cases with normal MF-PCR results.

Chapter 4
The association between specific histological features and cytogenetic abnormalities in 54 ectopic pregnancies was assessed. Histologic evaluation was carried out by two pathologists who were unaware of the cytogenetic outcome.

Chapter 5
Literature regarding chromosome abnormalities and miscarriages was reviewed. Subsequently, cytogenetic abnormalities, like numerical chromosome abnormalities, structural chromosome abnormalities and mosaicism, single-gene abnormalities and other genetic mechanisms were described. Special attention was paid to chromosome abnormalities in recurrent miscarriage.

Chapter 6
A cross-sectional survey was conducted to investigate policy on diagnosis and treatment of recurrent miscarriage in the Departments of Obstetrics and Gynecology in the Netherlands.

Chapter 7
A historical cohort study was conducted, including couples with recurrent miscarriage. Data was retrieved from medical records and telephone interviews. The obstetric follow-up was recorded for at least two years after parental karyotyping. Frequencies of carrierism for structural chromosome abnormalities in couples with maternal age <35 years, and maternal
age ≥ 36 years were compared at different points in time. A projected prevalence of
 carriership was calculated for these two subgroups by multiplying numbers of couples
 included to the original level of the screening population.

**Chapter 8**

The results of present studies are summarized. Implications for future research are given.


58 De la Rochebrochard E, Thonneau P. Paternal age and maternal age are risk factors for miscarriage; results of a multicentre European study. *Hum Reprod* 2002;17:1649-56.