Identify, appraise and individualize: clinical practice and prediction models in recurrent pregnancy loss

Youssef, A.

IDENTIFY, APPRAISE AND INDIVIDUALIZE: CLINICAL PRACTICE AND PREDICTION MODELS IN RECURRENT PREGNANCY LOSS

ANGELOS YOUSSEF

COLOPHON

Cover and illustrations: Iris Le Rütte
Lay-out: Niels Harlaar, Rogier Nell and Angelos Youssef

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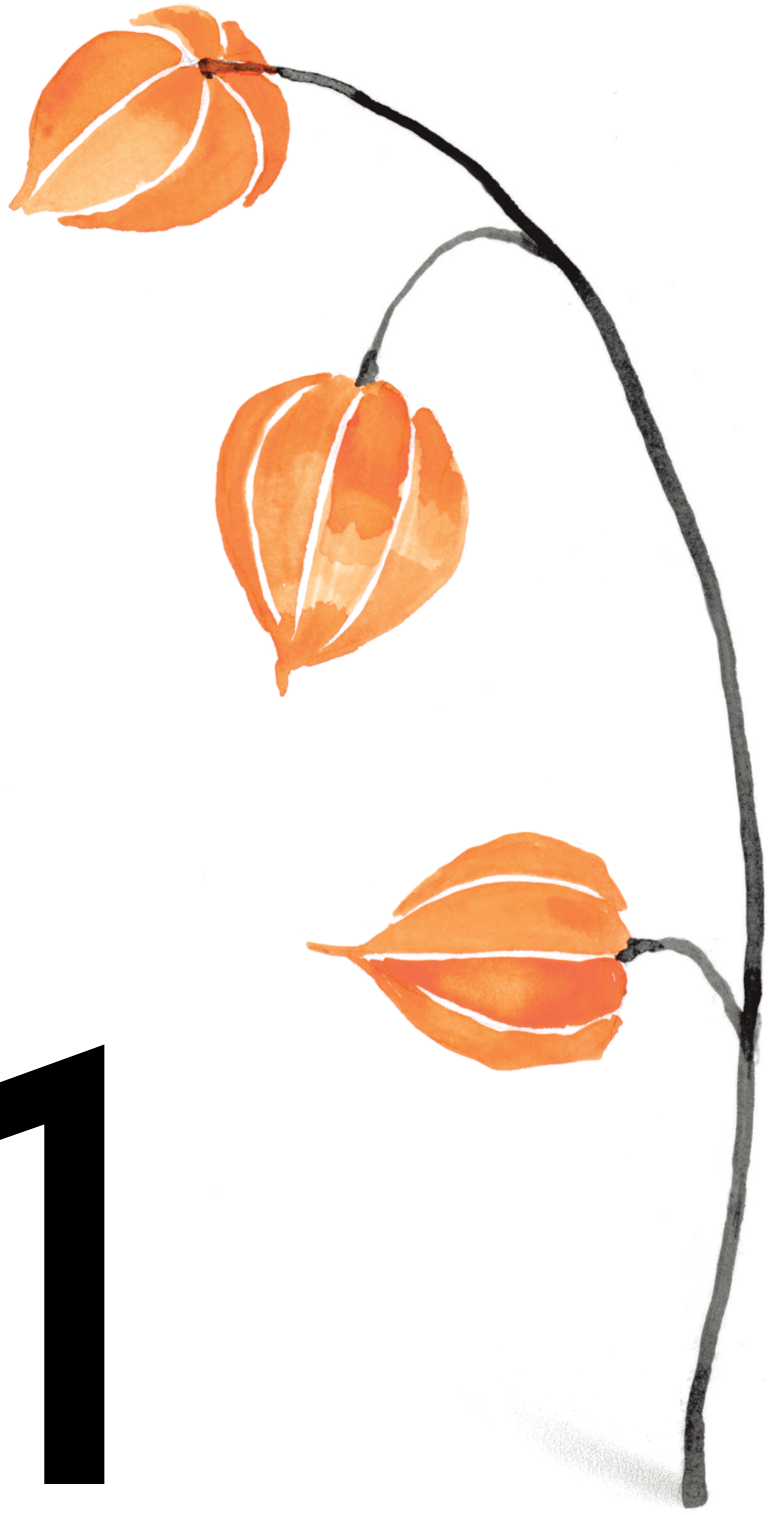
In de schaduw van verlies schijnt de kracht van ongeziene moed.
Aan hen die ongezien strijden tegen herhaalde miskramen.

THESIS OUTLINE



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CHAPTER 1

INTRODUCTION

INTRODUCTION

One of the most common complications of pregnancy is a miscarriage, defined as the spontaneous demise of a pregnancy before the foetus reaches viability. It can lead to great distress, especially when miscarriages are recurring. Recurrent miscarriages, or internationally defined as recurrent pregnancy loss (RPL), is a clinical disorder with several known underlying factors, although an identifiable factor is often not found. Today many questions are still unanswered regarding RPL, despite the shear amount of RPL studies published in the previous years. Foetal karyotyping of miscarriage tissue shows that more than half of the foetuses are aneuploid. It is thought that this aneuploidy is associated with increasing age, smoking and obesity, although couples with higher number of pregnancy losses tend to have more euploid pregnancy losses (3). The underlying mechanism of these miscarriages are currently not completely understood. Clinicians are therefore still unable to explain couples suffering from RPL why some pregnancies are successful and others are not.

As early as the 1930s, the first literature describing women with RPL started to emerge. Malpas described a population of women in which "sequential abortions" or "abortion sequences" could be identified (4). He hypothesized that two main groups exist, namely those who experience "sequential abortions" merely due to chance, and those due to recurrent factors. Using a mathematical equation assuming both accidental and recurring factors, he calculated that the proportion of pregnancy losses became almost constant after three consecutive pregnancy losses, regardless of the ratio of accidental and recurring factors. Ever since Malpas introduced the idea of "abortion sequences", there has been extensive discussion regarding RPL as a condition from both clinical point of view and from research. An agreement in defining, investigating and managing RPL is lacking, despite recommendations from recent guidelines, such as the guideline published by the European Society for Human Reproduction and Embryology (ESHRE). This is even more true for unexplained RPL, when an etiological factor cannot be identified, and treatment is not recommended (1). Differences in these aspects of RPL impact the level of evidence in this field.

The aim of this thesis is to identify variation in current RPL practice, to appraise existing evidence that could impact counselling of RPL couples in order to individualize RPL care and management.

RECURRENT PREGNANCY LOSS

EPIDEMIOLOGY

A substantial proportion of confirmed pregnancies are lost prior to live birth, making pregnancy loss the most common pregnancy complication, occurring in 10 to 15% of pregnant women (5, 6). This number could be even higher, as many conceptions are lost before women are aware of their pregnancy (7). Recurrence of pregnancy losses is much less common, the population prevalence of couples with 2 miscarriages is estimated as 1.9% (1.8–2.1%), for couples with 3 or more miscarriages it is estimated as 0.7% (0.5–0.8%) (5, 6, 8). The exact RPL incidence is however difficult to estimate. Both numerator and denominator are subject to uncertainty as it is difficult to estimate the number of couples that suffer from RPL at risk for RPL (all women at fertile age, or all women who try to get pregnant) (1). Moreover, these numbers are often derived from retrospective data in which selection bias is probable to occur. It is more likely that registry-based data could provide the true incidence of RPL. A substantial number of miscarriages however go unnoticed, as early pre-clinical pregnancies are often undetected. Furthermore, biochemical pregnancies are often not available in national registries. This means that women with non-clinical pregnancies do not visit healthcare professionals, and thus are not registered. Besides registry-based issues, time period plays an important role as well. Currently, family planning starts at older ages compared to previous generations (9). As age is an important risk factor to miscarriages, this could lead to a higher incidence of RPL. Furthermore, pregnancy tests are evolving and enable very early detection, leading to the ability to diagnose miscarriages that otherwise would not have been detected. And finally, a societal trend to discuss miscarriages has created more openness and probably more willingness to share information that could help clarify the exact magnitude of RPL (10).

DEFINITION

Although three pregnancy losses as definition for RPL is still being considered, defining RPL as two pregnancy losses onwards is currently increasingly being used. Actually, there is no consensus on the number of pregnancy losses, nor whether the losses have to be consecutive (11). Furthermore, the gestational age of pregnancy losses counted in the definition is still being discussed. The Royal College of Obstetricians and Gynaecologists (RCOG) defines RPL as three or more consecutive pregnancy losses (12), while the American Society for Reproductive Medicine Practice Committee defines RPL as two or more pregnancy losses confirmed by ultrasound or histology, not necessarily consecutive (13, 14). The most recent RPL guideline from ESHRE set the definition after a significant debate. It states that RPL could be considered after the loss of two or more pregnancies and stresses the importance of the need for further scientific research, including epidemiological studies on the effect of various RPL definitions on diagnosis, prognosis and treatment (15). Literature does not show differences in the prevalence of abnormal test results for the conditions associated with RPL (antiphospholipid syndrome (APS), uterine anomalies, thyroid disorders and chromosomal abnormalities), and therefore, there is no clear evidence of a pathophysiological difference between couples with two and couples with three or more pregnancy losses. Similarly, the consecutiveness of pregnancy losses does not seem to have a different pathophysiological pathway. As the burden of RPL on couples is aggravated by each loss, the argument for including two pregnancy losses in the definition could be made, knowing that timing of investigations does not play a role in finding underlying factors. On the other hand, the chance of a successful pregnancy after two losses is still very high. It is therefore debatable whether these couples suffer from an underlying condition. Knowing that these discussions will continue until epidemiological studies have unravelled the puzzle of defining RPL, the ESHRE argues that two or more pregnancy losses will facilitate research, shared decision-making and psychological support to couples.

RISK FACTORS

Multiple risk factors and underlying causes for RPL are known, and will be set out in the next paragraphs. It is important to understand the evidence-proven risk factors for RPL, in order to correctly identify and appraise differences in RPL literature. As the RPL definition varies between studies, so does the definition of unexplained RPL. It is important when comparing studies to understand what was meant by "unexplained" RPL, as this concept has evolved with time. Box 1 contains an overview of the known RPL risk factors.

Box 1 | Risk factors and etiological factors for recurrent pregnancy loss

- Maternal age
- Previous number of pregnancy losses
- Lifestyle factors (smoking, alcohol, stress)
- Abnormal parental karyotypes
- Congenital uterine anomalies
- Antiphospholipid syndrome (APS)
- Thyroid antibodies (thyroid peroxidase antibodies)
- Hypothyroidism
- Obesity
- Endometritis
- Male lifestyle factors

MATERNAL AGE

As is the case for women with sporadic pregnancy losses, age plays an important role (5, 16). The chance of a live birth decreases with increasing female age, and increasing female age is associated with more foetal chromosomal abnormalities (17-19). These abnormalities are present in most miscarriages (20).

PREVIOUS NUMBER OF PREGNANCY LOSSES

The number of previous pregnancy losses is a major risk factor for RPL. Age-adjusted odds ratios for pregnancy loss have been shown to increase subsequently after each pregnancy loss. Women with three or more pregnancy losses have an increased risk by a factor of 4.5 compared to women without pregnancy losses (3, 21-23). This relationship is possibly

explained by underlying factors (that may or may not be identified), due to which pregnancy losses keep occurring.

LIFESTYLE FACTORS

Although lifestyle factors such as smoking, alcohol consumption, as well as stress are not conclusively shown to cause RPL, they are associated with a negative impact on the chances of a live birth (15). Smoking and alcohol consumption are modifiable risk factors that are shown to increase maternal and foetal complications during pregnancy, and are shown to be dose-dependent. Although there is no direct evidence showing that these lifestyle factors cause RPL and there are no studies evaluating the role of smoking and/or alcohol cessation on live birth in RPL couples, it is established that these factors cause poor obstetric outcomes and are in general harmful. Obesity (BMI ≥30 kg/m^2) is an independent RPL risk factor, but overweight women are not at risk of RPL (15, 24, 25). Obesity is associated with other endocrine disorders, such as hypothyroidism, diabetes mellitus and Polycystic Ovary Syndrome (PCOS). These comorbidities however do not show a clear association with RPL, individually (26, 27).

GENETIC FACTORS

Couples with RPL show more translocations, inversions and copy number variations when compared to the general population (2–5% vs 0.7%). If one of the partners carries a structural chromosome abnormality, products of conception can have a normal karyotype, but could also have an unbalanced karyotype. It is known that unbalanced karyotypes can lead to miscarriage, stillbirth and major congenital malformations. In addition to the general association of unbalanced karyotypes, four susceptibility loci on chromosome 9 and 13 that play a role in placentation, gonadotropin regulation and progesterone production were identified in sporadic and recurrent pregnancy losses (28). A familiar component seems to play a role, as women with a history of pregnancy losses are more likely to have a family history of pregnancy losses, and siblings of women with RPL were found to have a twofold higher frequency of pregnancy loss compared to the general population (29, 30).

As mentioned, embryonic aneuploidy explains most of the pregnancy losses. The proportion of aneuploidy decreases from 60.9% to 24.4% in women with two pregnancy losses compared to women with six or more previous miscarriages (3). This could support the hypothesis that lower number of pregnancy losses could be more attributed to temporary unfavourable conditions, while higher number of pregnancy losses could be caused by permanent underlying conditions that are yet unknown.

CONGENITAL UTERINE ANOMALIES

Congenital uterine abnormalities are more prevalent in women with a history of pregnancy losses compared to the general population (13.3%; 95% CI 8.9–20.0% vs 5.5%, 95% CI 3.5–8.5%), with septate uterus being the most prevalent anomaly (31). Congenital uterine abnormalities more often lead to late first trimester and second trimester losses instead of early first trimester pregnancy losses (32). Acquired uterine abnormalities, such as uterine myoma's, might be associated with RPL, but the evidence is not clear (33).

AUTOIMMUNE DISORDERS

ANTIPHOSPHOLIPID SYNDROME

APS is an acquired thrombophilia syndrome characterized by vascular thrombosis and/or obstetric complications, including pregnancy losses, pre-eclampsia, preterm birth and foetal growth restriction. APS is the most prevalent treatable cause of RPL and is prevalent in 10–20% of RPL couples (23, 34, 35). Studies have suggested that the inflammatory effects of phospholipid antibodies caused by excessive complement activation affect placental and endothelial cells, however the precise mechanism that leads to obstetric complications is still unclear (36, 37).

ANTI-THYROID ANTIBODIES

Studies have found anti-thyroid antibodies more frequently in RPL couples compared to the general population, even in the absence of thyroid dysfunction (38, 39). Whether anti-thyroid antibodies cause RPL is unclear, as well as the mechanisms by which they possibly cause RPL.

ENDOCRINE FACTORS

THYROID FUNCTION

The prevalence of overt hypothyroidism seems higher in couples with RPL compared to a control group, as shown by one study, but the risk of RPL in hypothyroid women compared with euthyroid women was no different (40). High-quality evidence is lacking. Subclinical hypothyroidism however is not a risk factor for RPL (41).

DIABETES

Although the prevalence of diabetes in women with RPL is low, it is known that higher sugar-levels in the first trimester are associated with miscarriage, while women with well-controlled diabetes are not at risk (42, 43). The association between diabetes and RPL is unknown.

INHERITED THROMBOPHILIAS

Inherited thrombophilias such as Factor II and Factor V Leiden mutations, protein C, protein S and anti- thrombin deficiencies were marked as RPL risk factors in early studies. More recent analyses however have not confirmed these associations (44-46).

MANAGEMENT OF RPL COUPLES

Differences in the concepts of definition, investigations and treatments cause practice variations between clinics. This variation in RPL care is reflected in RPL couples wanting to go abroad for more extensive work-up and possible treatment, although evidence is often lacking for these practices. Cross border care has been studied in the context of reproductive care, and provided insight in its possible benefits and risks (47, 48). Although cross border care in the context of RPL has not been studied yet, clinical experience tells that RPL couples intending to go abroad often do it to undergo investigations and treatments that are not offered locally, mostly because these are deemed non-evidence-based. Offering such investigations and treatments goes in against the principle of "do no harm", as there is no foundation of evidence to perform them. The psychological and physical burden on the women is troublesome, and the burden on couples is aggravated with each further experienced pregnancy loss. It is understandable that they seek medical care to provide them with hope for their reproductive future. It is therefore important to provide RPL couples

with sufficient supportive care. In the following paragraphs, evidence-based and later non-evidence-based treatments will be discussed.

EVIDENCE-BASED THERAPIES

ANTIPHOSPHOLIPID SYNDROME

APS treatment for the improvement of live birth in women with RPL is recommended, and consists of heparin (low molecular weight or unfractioned) and low dose aspirin (15, 49, 50). Heparin alone has a lower efficacy than combined with low dose aspirin, and aspirin alone has no effect on live birth rates (50, 51). It is thought that heparin acts through its complement blocking action rather than its anticoagulant effect (52). The treatment regimen usually consists of starting low dose aspirin before conception, and adding heparin on the date of a positive pregnancy test. This regimen is continued throughout pregnancy until delivery (15). Although one of the criteria for APS is "three or more pregnancy losses", one study showed that there was no difference in the number of pregnancy losses between women with RPL and APS and women with unexplained RPL (53). Testing and treatment is therefore advised from two pregnancy losses onwards.

OVERT HYPOTHYROIDISM

Although evidence on the association between overt hypothyroidism and RPL is not conclusive, it is recommended to treat overt hypothyroidism with hormone replacement therapy as hypothyroidism is associated with adverse pregnancy complications and detrimental effects on foetal neurodevelopment (15). There is conflicting evidence on treatment of subclinical hypothyroidism in RPL couples, and there is insufficient evidence for hormone replacement therapy in euthyroid RPL women with thyroid antibodies (41, 54-56).

THERAPIES WITH LIMITED EVIDENCE

SURGICAL TREATMENT OF UTERINE ABNORMALITIES

During embryological development, incomplete fusion of Müllerian ducts results in an avascular uterine septum which divides the uterus into two cavities (57, 58). The goal of surgical treatment is to restore the uterine cavity by removing the septum to improve implantation. Reconstruction of these anomalies have been long debated in the context of improving live

birth in this group of RPL, but the results of a recent randomized trial (TRUST) showed that surgical interventions do not improve reproductive outcomes in women with septate uterus (59). Surgical interventions for other uterine anomalies are not recommended either (15).

MANAGEMENT OF UNEXPLAINED RPL

In the absence of identified RPL risk factors, some clinicians start empirical therapies, such as intravenous immunoglobulin (IVIG), aspirin and heparin (alone or combined), glucocorticoids and other therapies. These therapies that lack scientific base and are not supported in the management of unexplained RPL will be briefly discussed in the following paragraphs (15).

PROGESTERONE SUPPLEMENTS

The use of progesterone has been extensively studied for patients with unexplained RPL. Although a recent meta-analysis of 12 clinical trials suggested that there may be a reduction in the number of miscarriages for women given progestogen supplementation compared to placebo, this meta-analysis included 4 small studies with high risk of bias. The ESHRE guideline therefore bases its recommendation that vaginal progesterone does not improve live birth rates in women with unexplained RPL on the most recent and high-quality trial (60). Still, in recent trials a possible beneficial effect is shown for the use of progesterone in women with three or more pregnancy losses presenting with early bleeding in a next pregnancy (<AD16weeks) (61, 62). This was however a subgroup and requires further validation. One study showed that threatened miscarriage is associated with lower serum progesterone levels (63). The question remains however whether serum progesterone levels are low because of the pregnancy loss, or whether the pregnancy loss is caused by low serum progesterone levels. Further research is needed to clarify mechanisms by which progesterone improves pregnancy outcomes and to identify groups that can benefit from this treatment.

LYMPHOCYTE IMMUNIZATION THERAPY

Immunization with allogeneic lymphocytes of women with RPL is based on the hypothesis that women with RPL lack antibodies that protect the foetus from rejection by the woman (64). A systematic review however has

showed that no significant effect of lymphocyte immunization therapy is present on live birth rate in women with RPL (65).

INTRAVENOUS IMMUNOGLOBULIN THERAPY

Intravenous immunoglobulin (IVIG) therapy targets the reduction of symptoms in autoimmune diseases and has been studied in several trials in the context of improving live birth in couples with RPL (66). The studies were found to be underpowered and used different dosages and treatment regimens. Therefore, IVIG treatment cannot be recommended for clinical use in RPL care.

GLUCOCORTICOID TREATMENT

Treatment with glucocorticoids have a beneficial effect on auto-immune inflammatory diseases. It is hypothesized that women with RPL might have an immune aetiology and could therefore benefit from glucocorticoid therapy (67). Limited trials are preformed and although evidence points towards beneficial effects on live birth rate, adverse events appear higher in glucocorticoid treated patients (67, 68). It is therefore not recommended to use glucocorticoids as treatment in unexplained RPL, but new trials are needed to assess the effect lower doses of glucocorticoids.

ANTICOAGULANT TREATMENT

Although recent studies have shown that inherited thrombophilias are not associated with and RPL, anticoagulant treatment is still being administered to women with unexplained RPL. A Cochrane review studied live birth after anticoagulant therapy in women with RPL with or without thrombophilia and showed no beneficial effects regarding live birth rate for the studied anticoagulants (aspirin, heparin or a combination of both) (69). It is therefore not recommended to use anticoagulant therapy in women with unexplained RPL.

INTRALIPID THERAPY

Intralipids are intravenous lipid emulsions that are reported to lower Natural Killer cell cytotoxicity in women with recurrent implantation failure. The authors extrapolated these results and theorized that intralipids could be beneficial in couples with unexplained RPL (70). Although studies using low dosages have not reported serious adverse events, higher dosages

were associated with a series of a serious adverse events such as acute kidney and lung injury, and cardiac arrest (70-72). There is insufficient evidence to support intralipid treatment in RPL care.

ENDOMETRIAL SCRATCHING

Endometrial scratching has become popular prior to IVF interventions, and has been theorized to be of beneficial effect in embryo implantation. No trial has been performed so far in women with RPL and thus it is not recommended to be performed in RPL care.

THYROXINE TREATMENT IN EUTHYROID WOMEN WITH THYROID ANTIBODIES

Although pregnancy loss and thyroid auto-immunity have been associated, a recent systematic review and meta-analysis concluded that levothyroxine treatment in this group of women with RPL does not increase live birth rates and should thus not be recommended outside of clinical trials (41).

SUPPORTIVE CARE

Even though pregnancy loss is a common complication, it often has a negative impact on mental health. Understandably, the recurrence of these life events could further increase a negative impact. A cross-sectional study performed in Denmark showed that depression rates are higher in couples with RPL compared to healthy controls (73). International guidelines have therefore recommended specialised RPL units with dedicated specialists where supportive care could be offered to couples with RPL, focussing on both medical and psychological aspects.

Supportive care for women and their partners is thus central to the management RPL, especially in couples with no identifiable factor. Supportive care and intensive pregnancy surveillance in the first weeks of gestation are assumed to be of influence in the prevention of new pregnancy loss (74). It is important that individualized support should be provided in a dedicated clinic, that acknowledges pregnancy loss as a life event.

Part of this supportive care is counselling on the prognosis and live birth rate of subsequent pregnancies in couples with RPL. This information manages the expectations of the couple and improves their ability to make

an informed decision regarding further pregnancy attempts. There are two main prediction models implicated in RPL care, the model of Lund, et al., which is adapted by the American Society for Reproductive Medicine (ASRM), and the model of Brigham, et al., which has been implicated in RPL care in the Netherlands and the United Kingdom (12, 14, 75, 76). These models provide an estimate of a successful pregnancy, based on age and number of previous pregnancy losses. Although these models were both reviewed with high methodological quality by the ESHRE and both studies have consistent results, these models did not follow the nowadays recommended TRIPOD guideline in the development and reporting of a prediction model, which assures transparent reporting, and acts as a tool for reminding authors of all necessary prediction components, such as measuring the predictive performance of the study internally and/or externally (15, 77).

To be able to deliver the best possible (supportive) care, it is important to be aware of the latest evidence regarding RPL. Guidelines and their updates are usually published every so many years, but implementation of these guidelines that summarize the latest evidence and recommendations that follow from it often lacks, leading to large RPL practice variation. The ESHRE practice guidelines for example are intended to maximize implementation at clinics, by following an extensive procedure consisting of evidence review, quality assessment and translation of a set of best practice recommendations (78). Besides implementation, dissemination and promotion of guidelines should increase awareness of medical professionals about new practice recommendations. The ultimate goal of this process is to reduce inappropriate practice variation (78). A recently conducted ESHRE study however concluded that implementation strategies are lacking and that on average only one-fourth of clinicians that are aware of new guidelines go on and make changes in their routine practice (79). It is therefore important to understand the magnitude of RPL practice variation, and to study barriers for guideline implementation in order to reduce inappropriate practice variation. This is especially the case for the Dutch RPL guideline, which has not been updated since 2007 and is currently undergoing revision.

PREDICTION

Prediction models are tools that aim to provide information about the likelihood of uncertain outcomes (80). Prediction models combine characteristics of individual patients to predict the presence of a disease or a future outcome. The use of these models aims to assist clinicians and patients with informed decision making, based on individual profiles of prognostic factors. There are four key steps to making prediction models useful: development, validation, impact evaluation and model updating (81).

Usually, prediction models are derived from multivariable regression models for dichotomous outcomes. In the development phase, missing data, prognostic factors and model complexity should be addressed (82, 83). It is important for the development dataset to be large enough to develop a model that is reliable when applied to new individuals. In other words, the sample size needs to be large enough to avoid overfitting (84). An established rule of thumb is to ensure at least 10 events for each predictor parameter being considered for inclusion in the prediction model (84). The adequacy of this rule of thumb has been debated, as the required events per predictor parameter is context specific and relies on a number of factors. A four-step procedure was therefore developed by Riley, et al. to ensure a precise estimate of the overall outcome risk, a small mean error for predicted values across all individuals, a small required shrinkage of predictor effects and a small optimism in apparent model fit (84).

When evaluating the predictive ability of the model on the same data that was used to develop the model, overfitting will lead to an optimistic estimate of model performance. This occurs when too few events relative to the number of selected predictors are included (77). It is therefore important when developing a new prediction model to include internal validation. This quantifies the optimism in the performance of the prediction model, and adjusts it for overfitting (77). Methods such as cross-validation and bootstrapping are often used as internal validation techniques, which is a necessary step in prediction model development (81).

Prediction models need to be validated with patient data that was not used for the development process, as it is most probable that the original model

will show optimistic results (80). By validating the original model in a different dataset, model generalizability can be confirmed. External validation consists taking the original model with its predictors and assigned weights and applying them in new individuals, after which the predictive performance of the model can be quantified (85). The performance of a prediction model is typically measured in two dimensions, namely calibration and discrimination. Calibration analyses the agreement between the predicted and observed pregnancy success rates, while discrimination examines the ability of the model to separate between women with a successful pregnancy and those without (85).

When prediction models are implemented in clinical management, their impact should be evaluated as patient outcomes and cost-effectiveness of clinical practice could be influenced (86, 87). Prognostic models have a cost in their implementation and might even have adverse consequences on clinical outcomes if they lead to decisions that withhold beneficial treatments.

Lastly, it is desirable to update the prediction model, as systemic miscalibration is common when models are developed in a different setting (80). Updating models include recalibration, or investigating the addition of new prognostic factors (81).

THESIS OUTLINE

In an attempt of improving the quality of RPL care, this thesis will focus on two main areas. In Part I, the existence of various guidelines and their use in clinical practice are central in understanding current day RPL care. It is important to study (inter)national differences in RPL care, as high levels practice variation might lead to an increasing number of patients seeking care in various (inter)national centres, to be offered more extensive investigations and various non-evidence-based treatments. By studying possible barriers in implementing evidence-based guideline and adapting implementation strategies to avoid these barriers, new tools can be developed to help overcome these barriers. Part II of this thesis focusses on the use of prognostic tools in supportive care for RPL couples. As counselling on future pregnancies plays a key role in RPL practice, it is important that clinicians provide accurate information. After reviewing available prognostic tools and evaluating them, a new prognostic tool will be introduced.

PART I – GUIDELINES AND IMPLEMENTATION

In **chapters 2 and 3**, national and international RPL guidelines are studied regarding definitions, investigations and treatments offered to couples with RPL.

In **chapter 4**, results of a nationwide survey conducted under medical professionals linked to RPL care are presented. In this survey, barriers for the implementation of a new evidence-based RPL guidelines in the Netherlands are identified.

In **chapter 5**, results of an implementation strategy to improve adherence to the RPL guideline are presented. Various tools are suggested for consideration to be used in future guideline implementations.

PART II – PROGNOSIS IN RECURRENT PREGNANCY LOSS

In **chapter 6**, differences and similarities between couples with two and three or more pregnancy losses are explored in an effort to provide insight in the discussion how RPL should be defined. Additionally, prognostic details of the studied cohort are presented.

In **chapters 7 and 8** we focus on the role of prognostic counselling in couples with RPL. The current literature of prognostic models providing individual risk assessment for future pregnancies in couples with RPL are studied and evaluated in chapter 7. In chapter 8, we zoom in on one of the most widely used prognostic models that predict the live birth chance in couples with RPL in the first pregnancy after intake.

In **chapter 9**, we conclude the prognosis part of this thesis by presenting a study protocol that aims to predict pregnancy success in couples with both unexplained and explained RPL, involving both prospective and retrospective cohorts (OPAL-prediction model).

Finally, in **chapter 10** presents a summary of all studies included in this thesis, followed by a general discussion and recommendations for future research.

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PART I

GUIDELINES AND IMPLEMENTATION

2

CHAPTER 2

PORTRAYING CLINICAL VARIATIONS: RECURRENT PREGNANCY LOSS GUIDELINE

A. Youssef, E.E.L.O. Lashley, M.L.P. van der Hoorn

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INTRODUCTION

In the "portraying clinical variations" column, differences and similarities between various local and regional protocols and national guidelines of a common disease are investigated. For this a sample of academic and non-academic protocols, geographically dispersed throughout the Netherlands, is used. This column is written by two obstetrics & gynaecology residents, with the aim of discussing the unification of local and regional protocols in accordance with national guidelines.

In prior editions of this protocol discussion, the protocols gestational diabetes and hypertensive disorders in pregnancy have been discussed. This third part of the series discusses the recurrent pregnancy loss (RPL) guideline (1, 2). Seven local protocols will be compared to each other, as well as to the national guideline (3). Besides the seven approached hospitals, one academic and one non-academic hospital indicated that they work with the national guideline without local adjustments.

DEFINITION OF RPL

RPL is defined as the objectified loss of two pregnancies of a couple. The losses do not have to be consecutive. Ectopic pregnancies and molar pregnancies are excluded from the definition.

NATIONAL GUIDELINE

The Dutch national guideline of the Dutch Society of Obstetrics and Gynaecology (NVOG) dates from 2007. This guideline discusses the probable risk factors of RPL. Furthermore, it provides an overview of recommendations regarding investigations and therapy, based on available literature. In summary, there are no recommended drug treatments (except for antiphospholipid syndrome (APS)); lifestyle recommendations are the main treatment options.

RPL

Approximately 15% of pregnant women experience a pregnancy loss. Only 3% of pregnant women experience the loss of two pregnancies, and 0.4 – 1 percent experience the loss of three pregnancies. There are two key concerns in couples with RPL: the cause of RPL and the chance of recurrence. In a first pregnancy, the chance of a pregnancy loss in the

following pregnancy remains 15% (depending on a maternal age), but rises to 17-31% after two pregnancy losses and up to 46% after three pregnancy losses. For that reason, investigations after two pregnancy losses are recommended (4-6). Unfortunately, in up to 50% of couples no cause can be identified (7). The chance of an ongoing pregnancy after RPL depends on maternal age, and the number of preceding pregnancy losses. In general, the chances of a successful pregnancy are higher than a negative outcome. The chance of an ongoing pregnancy beyond 12 weeks is approximately 75% in a next pregnancy (8).

GENERAL IMPRESSIONS OF GUIDELINES

Besides the fact that protocols should be practical and effective in daily clinical practice, there are a couple of other points to assess the general quality of the guidelines.

1. Is the intended population clearly defined and complete?

2. Are the recommendations for diagnostics, treatment and follow-up clear? In other words: not highly subject to individual interpretation or free of interpretation?

3. Do the given recommendations match the best available evidence and/or national guideline? Are there references to used sources?

4. Does the protocol only apply at the clinic or is it a regional or transmural protocol?

Mentioned in table 1 are the protocols from different (anonymised) academic and non-academic hospitals. Except protocol P3, all protocols mention their definition for RPL and all protocols clearly define the targeted population. The protocols vary in terms of information provision, some of the protocols provide brief background information and touch briefly upon investigations and therapy. Other protocols contain extensive background information and specify per probable cause the therapeutic options. The Dutch guideline is seen as a guide by all protocols, there are however a few differences. These differences will be explored below. The protocols relate to a specific clinic and are not transmural. Protocols U2 and P4 provide a thorough reference list, U1, P1, P2 and P3 only refer to the NVOG guideline.

RESULTS

TERMINOLOGY

In 2005, the Special Interest Group for Early Pregnancy of the European Society for Human Reproduction and Embryology (ESHRE) suggested to change the European nomenclature of young pregnancies in the English language (9). Reason was that the old terminology dated from before the ultrasound era. Furthermore, different terms were being used in describing the same phenomenon, which could lead to confusion. In 2008, the NVOG suggested to replace "habitual abortion" with "recurrent pregnancy loss" to prevent confusion in patients and in medical staff (10). Except for protocol P3, the definition of RPL is mentioned in each protocol and is generally speaking in accordance to the NVOG definition.

DIAGNOSTIC DECISION AID

One protocol (P3) refers to the decision aid found on the Freya website. This program is based on the Dutch guideline and provides medical staff the ability to determine which investigations should be performed for each RPL couple (11).

RISK FACTORS

Five of the seven protocols contain information regarding risk factors. In the remaining two protocols (U3 and P3), risk factors are mentioned in the paragraphs describing treatment options. What stands out here is the mentioning of M. Wilson as a risk factor in protocol P2, while this risk factor is not mentioned by the NVOG. M. Wilson is a very rare, autosomal recessive disease occurring in 1:30.000 live births (12). Subfertility is followed by menstrual abnormalities caused by liver cirrhosis. Spontaneous pregnancies in untreated disease, will result in pregnancy losses through the excessive copper amounts in the uterus (13-15). The treatment of this disease is mentioned in the treatment paragraph of this protocol, as well as in protocol P1. The latter protocol however did not mention this disease as a risk factor.

INVESTIGATIONS

GENERAL

The protocols state in varying manner which components of the patient history are important. Four protocols (U1, U2, U3 and P2) clearly state what questions should be included when obtaining a patient's history. Other protocols do not mention this, or mention it very briefly.

GENETICS

Karyotyping is being performed when a risk assessment based on age and family history provides reason to do so. The chance of structural chromosomal abnormalities in couples with two or more pregnancy losses is key here. According to the NVOG, it is advised to perform karyotyping in women when 34 years of age and younger at time of the second pregnancy loss, regardless of the total number of pregnancy losses is. Between the age of 34 and 39 at time of the second pregnancy loss, it is advised to perform karyotyping depending on family history, total number of pregnancy losses and maternal age. In women above 39 years old, it is advised not to perform genetic testing. All protocols mention karyotyping, only one protocol (P2) does not refer to the risk assessment table available in the NVOG guideline. The protocol of an academic hospital (U3) shows that karyotyping is also performed in case of a patient history with genetic abnormalities, or family genetic abnormalities.

It is known that 50% of early pregnancy losses are caused by chromosomal abnormalities of the foetus. Numerical abnormalities have no increased risk for a new pregnancy, karyotyping thus has no clinical consequences. The NVOG advises not to perform genetical testing on the pregnancy product. Protocol U1 mentions that this could be performed in case of scientific research.

THROMBOPHILIA

Antithrombin III, Factor II, V Leiden and Protein C and S are investigated on indication in all hospitals. Protocol P2 does not mention that screening is performed in case of a patient history or family history of thrombophilia as mentioned by the NVOG. The screening performed by all hospitals largely matches each other. Only protocol U1 performs extensive coagulation testing in case of three pregnancy losses.

IMMUNOLOGY/ANTIPHOSPHOLIPID ANTIBODIES SYNDROME

These investigations are focused on detecting APS. Anticardiolipin antibodies (ACA) and Lupus antibodies (LAC) should be tested at least twelve weeks after the occurrence of a pregnancy loss, followed by a confirmation test also twelve weeks later. All of the protocols perform the investigations as mentioned by the NVOG. In four hospitals (U1, U2, P2 and P4) bèta-2-glycoprotein is also tested for. The protocols do not always mention what type of antibodies are tested for (IgG and IgM). There's a difference in the advice of the timing of testing for APS. This ranges from six to twelve weeks after pregnancy. Two of the seven protocols do not advice on repeating testing.

HYPERHOMOCYSTEINEMIA

The protocols use different investigational methods to assess hyperhomocysteinemia. These methods are: random homocysteine, fasting homocysteine and methionine load test. The methionine load test can be performed in patients where hyperhomocysteinemia is suspected, but fasting homocysteine is not outside normal values. All academical hospitals use random homocysteine measurement.

ENDOCRINOLOGY

Endocrinological investigations are not discussed in protocols P1, P2 and P3. The other protocols describe the measurement of thyroid function only in context of the T4LIFE study, for which TPO antibodies are also investigated, or in the context of a patient history suggesting thyroid disease. It is known that TPO antibody levels could increase the chance of pregnancy loss and preterm birth. The T4LIFE study investigates whether levothyroxine in patients with normal thyroid function but TPO antibodies and RPL increases the live birth rate (16).

ANATOMY

Ultrasound of the uterus is advised in 5 protocols (U1, U2, U3, P2 and P4). One academic protocol, U1, uses 3D ultrasonography only in case of suspicion of a uterine anomaly.

THERAPY

LIFESTYLE RECOMMENDATIONS

Protocols U1, U2, U3, P1 and P4 contain lifestyle recommendations according to the NVOG guideline. Protocol P4 also advises to reduce coffee and alcohol intake and to check the usage of medications. Protocol P2 does not specify the recommendations, protocol P4 only notes that weight loss should be advised. These recommendations are for all patients, independent of underlying disease.

ANTIPHOSPHOLIPID ANTIBODIES SYNDROME

Except for two protocols, APS treatment is as advised by the NVOG guideline: 75-80 mg aspirin preconceptionally until 36 weeks gestation in combination with low molecular weight heparin once daily from the detection of a foetal heartbeat. Protocol P2 advises 5000-E-heparin twice daily. Protocol P4 advises starting fraxiparin once there's a positive pregnancy instead of at the detection of foetal heartbeat. Academic protocol U1 refers to their own APS protocol.

The evidence and effectivity of the treatment described above is not of high quality. The NVOG refers to two systematic reviews and an RCT, concluding that the combination of heparin and aspirin reduce the chance of a pregnancy loss (17-19). The national antithrombotic guideline recommends considering this treatment regimen (20). A recent meta-analysis concludes that this treatment is not effective (21). Furthermore, it is concluded that aspirin without heparin results in negative outcomes. In other cohort studies this negative association between aspirin and the risk of pregnancy loss was already made (22).

The suggested mechanism described is that aspirin suppresses the biosynthesis of prostaglandin, which plays an important role in the implantation of the embryo. Regarding the order of starting aspirin and heparin, there is no recommendation described in scientific literature. Expert opinion advises to start with heparin at time of a positive pregnancy test, followed by aspirin at detection of a foetal heartbeat because of the assumption that heparin plays an immunological role in the implantation.

BALANCED CHROMOSOMAL ABNORMALITIES

In case of a balanced chromosomal abnormality, the three academic hospitals advise referring the couples to a geneticist. Protocols P1 and P3 do not describe a treatment proposal, in other protocols it is described that couples should be counselled and should be offered antenatal investigations in the following pregnancy. Four of the seven protocols mention the possibility of preimplantation genetic testing after IVF, as mentioned in the NVOG guideline.

SEPTATE UTERUS

Treatment of a uterine septum is being investigated in the TRUST study, which is an RCT investigating the effect of hysteroscopic resection of the septum on live birth rate (23). This study is mentioned in U1, U2 and P4

THROMBOPHILIA

In case of thrombophilia, U1 and U3 refer to the ALIFE-II study, which investigates the effect of low molecular weight heparin on the chance of a pregnancy loss in comparison with no treatment (24). The other protocols do not contain any treatment, based on the lack of evidence.

HYPERHOMOCYSTEINEMIA

The Dutch guideline recommends investigating folic acid, vitamin B6 and vitamin B12 in case of abnormal homocysteine levels. Homocysteine supplementation is recommended at low levels, after which homocysteine measurement should be repeated 6 weeks after supplementation. The Dutch guideline also suggests when trying to get pregnant could be considered responsible, and how to manage during and after pregnancy. This management is based on the Dutch Health Council (25). Two academic protocols and two non-academic protocols (U1, U2, P3 and P4) are mostly the same as the NVOG. The last academic protocol does not recommend anything regarding hyperhomocysteinemia treatment in a following pregnancy. The two other non-academic protocols touch very briefly upon folic acid and vitamin B6 supplementation.

MORBUS WILSON

M. Wilson or hepatolenticular degeneration usually results in neurological symptoms, psychiatric symptoms or symptoms of liver failure. RPL is

almost never a first symptom (26). Two non-academic hospitals mention the treatment of the disease, P1 with penicillamine or zinc and P2 with penicillamine. M. Wilson was not mentioned as risk factor in protocol P1. Penicillamine binds copper an excretes it through urine. Because of its side effects, trientine can be used (27). There are no trials comparing these two chelators.

UNEXPLAINED RPL

Two of the three academic protocols (U1 and U3) mention the practice of Tender Loving Care (TLC) in unexplained RPL. They offer weekly ultrasounds during pregnancy and offer psychological counselling. One non-academic hospital also mentions TLC options in unexplained RPL. Almost all protocols refer to the high success rates in a following pregnancy mentioned in the Dutch Guideline.

CONCLUSION

In this edition of "Portraying clinical Variations", seven protocols have been compared to each other and to the NVOG guideline. The differences between protocols were merely in the details of performed investigations. Not always the protocols mention when to perform what investigations. This could result in confusion for residents, as it is important for example for some investigations to be performed several weeks after a pregnancy. Descriptive information can be useful in making the choice for investigations understandable. In many hospitals, a specialist outpatient clinic for RPL couples is available. Most of the time, this clinic is run by a small team, it is of interest for the resident to be able to witness and participate in this consultation. It could also be that patients visit general outpatient clinics, with a history of two or more pregnancy losses. Knowledge of RPL protocols is of importance for the resident, as they could provide information regarding RPL for each couple. A well-designed guideline is desirable. Most of the guidelines are well designed, but some are obsolete, as they did not include the results of the Promise trial (28). This is also the case for the national guideline, which dates to 2007. It is therefore time to revise the guideline. There are however several studies that investigate outcomes in couples with RPL. Thus, it is sensible to wait for those results, after which a new version of the guideline could be developed.

TABLE 1 | RPL practice across academic and non-academic hospitals

	NVOG guideline	Academic Hospital U1	Academic Hospital U2	Academic Hospital U3
Terminology	Recurrent Pregnancy Loss	Recurrent Pregnancy Loss	Recurrent Pregnancy Loss	Recurrent Pregnancy Loss
Defining RPL	2 or more objectified pregnancy losses (could be non-consecutive)	Conform NVOG	Conform NVOG	Conform NVOG
Risk factors	- APS - Cytogenetic abnormalities - Embryonic factors - Endocrinologic - Hyperhomocysteinemia - Living habits - Maternal Age - Thrombophilia - Uterine factors	- Cytogenetic abnormalities - Endocrinologic factors (Anti-TPO, disturbed glucose balance) - High Maternal Age - Hyperhomocysteinemia - Living habits (smoking, obesity) - Clotting abnormalities (thrombophilia, APS) - Uterine factors	- Previous pregnancy losses - Endocrinologic factors - Genetic factors - Hematologic factors - Immunologic factors - Living Habits - Maternal age - Paternal factors - Uterine factors	- Not specified
Investigations				
General investigations	- BMI - Living habits	- BMI - Living habits - Extensive patient history	- BMI - Living habits	- BMI - Living habits - Extensive patient history
Genetics	- Karyotyping according to risk table - No genetical testing of foetal material	- Conform NVOG - Karyotyping of foetal material only in scientific research setting	- Conform NVOG	- Conform NVOG + - In case of family history of chromosomal abnormalities

Genetics (continued)				- Previous child with genetic abnormalities - In case of consanguinity
Thrombophilia	- Only in case of venous thromboembolic event in patient history and/or first-degree family with thrombophilia and venous thromboembolic event - Antithrombin III - Factor V Leiden - Factor II - Factor VIII - Protein C and S	- After two pregnancy losses: conform NVOG - After 3 pregnancy losses: - Antithrombin activity - APC-resistance, in case of abnormal value Factor V Leiden analysis. - APTT - Factor II mutation - Factor VIII - Fibrinogen - INR - Lupus APTT - Protein C-activity and antigen - PT - Thrombin time - Free proteins S-antigen	- Conform NVOG	- Conform NVOG - - Factor VIII not mentioned
Immunology/APS	- ACA IgG - ACA IgM - LAC	- Conform NVOG + - Anti β2-glycoprotein IgM - Anti β2-glycoprotein IgG - Value range given - Investigations performed 8 weeks after pregnancy loss	- Conform NVOG - Value range given - Investigations performed 12 weeks after pregnancy loss	- Conform NVOG + - Anti β2-glycoprotein IgM - Anti β2-glycoprotein IgG - No value range given - Investigations performed 12 weeks after pregnancy loss
Hyperhomocysteinemia	Random homocysteine	Random homocysteine	Random homocysteine	Random homocysteine

Endocrinology	Not indicated	TPO-antibodies in case of T4LIFE participation If patient history suggests abnormalities: Glucose, HbA1C, TSH, thyroxin (T4)	Thyroid functions in case of T4LIFE participation	TSH and TPO-antibodies In case of abnormal TSH values, Free T4 T4Life
Anatomy	No recommendation	3D ultrasound in second half of menstrual cycle in case of suspected anomaly, unless patient carried pregnancy until term. In case of suspected cavity anomaly: sonohysterography.	Transvaginal ultrasound	Ultrasound of uterus cavity In case of abnormalities, sonohysterography or hysteroscopy
Therapy				
Lifestyle recommendation	Weight loss Healthy diet Smoking cessation Vitamin supplementation not useful	Conform NVOG	Conform NVOG	Conform NVOG
APS	Aspirin 75-80 mg once daily preconceptionally until 36 weeks gestation together with LMWH subcutaneously once daily following detection of foetal heartbeat until delivery	Referral to local APS protocol	Conform NVOG	Conform NVOG

Balanced chromosomal abnormalities of the couple	IVF + preimplantation genetical testing	IVF and preimplantation genetical testing explanations Consulting clinical geneticist	Referral clinical geneticist Invasive diagnostic testing with regard to the low chance of live born children IVF + preimplantation genetic testing	Consult clinical geneticist
Septate uterus	Surgery not recommended	TRUST study	Surgery only in case of TRUST study participation	Ultrasound of uterus cavity In case of abnormalities, sonohysterography or hysteroscopy
Thrombophilia	No evidence for anticoagulatory drug treatments	ALIFE II study Treatment according to haematology protocol	ALIFE study Anticoagulatory treatment according to protocol	Referral to obstetrician
Hyperhomo-cysteinemia	Measurement of fasting total homocysteine level at least 12 weeks after pregnancy Measurement of fasting total homocysteine level at least 12 weeks after pregnancy Based on the results, vitamin supplementation can be recommended, after which homocysteine levels can be measured again after 6 weeks	Conform NVOG	Conform NVOG	Measurement of fasting total homocysteine level at least 12 weeks after pregnancy Measurement of fasting total homocysteine level at least 12 weeks after pregnancy Based on the results, vitamin supplementation can be recommended, after which homocysteine levels can be measured again after 6 weeks

M. Wilson	Not specified	Not specified	Not specified	Not specified
Unexplained RPL	Tender loving care: Smoking cessation Early pregnancy ultrasound Psychological support	Tender loving care: Approachable team Weekly ultrasound until 12 weeks gestation Psychological support	Not specified	Frequent ultrasound if desired
Success rate following pregnancy	Table	Table NVOG	Table NVOG	Table NVOG
Follow Up	Guidance throughout first trimester	Weekly ultrasound until 12 weeks gestation	Preconception consults for early ultrasound	Not specified
Research		ALIFE II, REMI, T4 life, TRUST	Not specified	ALIFE II, T4 life, TRUST
References		NVOG guideline and websites	Very thorough	None

	Non-academic hospital P1	Non-academic hospital P2	Non-academic hospital P3	Non-academic hospital P4
Terminology	Recurrent Pregnancy Loss	Habitual abortion or recurrent pregnancy loss	Habitual abortion	Recurrent Pregnancy Loss
Defining RPL	Conform NVOG	Habitual (>3 consecutive spontaneous abortions) Recurring (>2 consecutive spontaneous abortions)	Not specified (Webtool website Freya)	Conform NVOG
Risk factors	- Cytogenetic abnormalities - Family history - Maternal age - Obstetric history	- Deficiency of: - Antithrombin III, Protein C, Protein S, APC-resistance with or without Factor V Leiden mutation, spontaneous thrombocyte aggregation. - Balanced chromosomal abnormalities - Hyperhomo-cysteinemia - Maternal age - M. Wilson - Thrombophilia - Uterine abnormalities	- Not specified	- Anatomical abnormalities - Chromosomal abnormalities - Endocrine abnormalities - Hyperhomo-cysteinemia - Lifestyle - Unknown - Thrombophilia
Investigations				
General investigations	Conform NVOG	Karyotyping of both parents	Conform NVOG	Conform NVOG

Genetics	Conform NVOG	Karyotyping of both parents	Conform NVOG	Conform NVOG
Thrombophilia	Conform NVOG + APC-resistance	- APC-resistance - AT III - Factor II variant - Factor V Leiden - Factor VIII - Protein C and S - Thrombocyte count	Conform NVOG	Conform NVOG
Immunology/APS	- ACA - LAC - Measured 6 weeks after pregnancy loss	- ACA IgM and IgG - Anti β2-glycoproteïne I - Measured 8-10 weeks after pregnancy loss	- Conform NVOG - Measured 12 weeks after pregnancy loss (2x measured)	- ACA - Anti β2-glycoprotein antibodies - LAC - Measured 12 weeks after pregnancy loss (2x measured)
Hyperhomocysteinemia	Methionine load test	Fasting homocysteine	Fasting homocysteine	Homocysteine
Endocrinology	Not specified	Not specified	Not specified	Thyroid function and glucose (in case of complaints)
Anatomy	Not specified	Uterine ultrasound	Not specified	Uterine ultrasound or hysteroscopy

Therapy				
Lifestyle recommendation	Conform NVOG	Not specified	Weight loss	Weight loss Smoking cessation No excessive coffee and alcohol intake. Focus on medication use (NSAIDs), job and socio-economic status
APS	Conform NVOG	2x 5000 E heparin and 75 mg aspirin per day	Conform NVOG	Aspirin conform NVOG Once daily LMWH subcutaneously following positive pregnancy test Consider starting aspirin following a positive pregnancy test, especially in case of subfertility
Balanced chromosomal abnormalities of the couple	Not specified	Counselling, antenatal investigations in next pregnancy	Not specified	Preimplantation genetical testing according to risk of chromosomal abnormalities (NVOG table)
Septate uterus	Not specified	No evidence for surgical removal	Not specified	Surgical removal only in case of TRUST study participation
Thrombophilia	Not specified	In consultation with haematologist	In consultation with haematologist	Conform NVOG Vademecum Haematology

Hyperhomocysteinemia	Folic acid 5 mg and vitamin B6	Folic acid and/or vitamin B6	Conform NVOG - Homocysteine investigation three months after pregnancy not mentioned	Conform NVOG
M. Wilson	Penicillamine of zinc	Penicillamine	Not specified	Not specified
Unexplained RPL	General comment: treatment is only indicated when proven to reduce chance of a new pregnancy loss significantly	Not specified	Not specified	Psychosocial support Frequent ultrasound Lifestyle advice RPL outpatient clinic Participation in studies No anticoagulant therapy
Success rate following pregnancy	Not specified	Table NVOG	Table NVOG	Table NVOG
Follow Up	Not specified	Not specified	Not specified	No second line care, but increased risk of maternal and foetal complications (preeclampsia, placental abruption, PPROM, preterm birth, foetal growth restriction)
Research	Not specified	Not specified	Not specified	Promise, TRUST, REMI, ALIFE2, T4Life
References	NVOG guideline	NVOG guideline	Freya website NVOG guideline	Very thorough

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CHAPTER 3

COMPARISON AND APPRAISAL OF (INTER)NATIONAL RECURRENT PREGNANCY LOSS GUIDELINES

A. Youssef, N. Vermeulen, E.E.L.O. Lashley, M. Goddijn, M.L.P. van der Hoorn

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ABSTRACT

Investigations and treatment options of recurrent pregnancy loss (RPL) differ internationally. This manuscript reviews the similarities and differences between international guidelines. The European Society of Human Reproduction and Embryology (ESHRE) guideline (2017), the American Society for Reproductive Medicine (ASRM) Committee Opinion (2013) and the Royal College of Obstetricians and Gynaecologists (RCOG) guideline (2011) were appraised using the AGREE II criteria. The guidelines were checked for definitions, risk factors, investigations and therapeutic options. The guidelines agreed on acquired thrombophilia analysis. All guidelines agreed on a regimen for the treatment of antiphospholipid antibody syndrome consisting of aspirin and heparin, but only the ESHRE guideline specified the order of starting these medications. Treatment of thrombophilia and uterine anomalies was advised against; all guidelines recommended supportive care for unexplained RPL. The guidelines did not agree on the definition of RPL and differed in investigations regarding lifestyle, karyotype analysis of parents and/or pregnancy tissue, and the diagnostic tool for uterine anomalies. All three guidelines indicate an association between lifestyle and RPL; the ESHRE recommends health behaviour changes. Couples suffering from RPL should be informed about possible investigations and treatment options, and whether those are evidence-based. It is important for clinicians to realize that the guidelines differ internationally.

INTRODUCTION

The first literature describing women with recurrent pregnancy loss (RPL) dates back to the early 1930s. Malpas described a population of women in which miscarriage sequences, at that time named 'abortion sequences', could be identified (1). A model was developed which concluded that three consecutive miscarriages was more than just bad luck (1). The first definition of RPL was three or more consecutive early pregnancy losses prior to the 20th week of gestation, which at that time was referred to as 'repeated early spontaneous pregnancy wastage' (2). Over the years, many underlying risk factors and treatment options have been described and criticized. Not only are the risk factors and treatments debatable components of RPL, but also there is no international consensus regarding the definition.

We recently compared the national protocols on RPL of three academic hospitals and four general hospitals in the Netherlands (3). Differences between diagnostic investigations and treatment options were the most common discrepancies between the protocols. Thus, in addition to an internationally varying definition, we have seen that despite the presence of a national guideline (4), local protocols are inconsistent.

In an attempt to understand this variation in clinical practice, this paper will summarize the similarities and differences between international guidelines on RPL. This exercise is also able to detect areas of uncertainty and evidence gaps and provide information as to which studies should be carried out to improve future RPL care.

It is important to be aware of the (inter)national differences in the care of RPL couples. In our own experience, patients frequently ask for a second opinion in another clinic, or even go abroad. These couples most often then undergo more extensive diagnostic testing, and are being offered treatment options that lack scientific rationale. There is an increasing range of tests and therapies offered to women attending RPL clinics. In general, practice variation can be resolved by the implementation of evidence-based guidelines. This paper provides an overview and critical appraisal, and compares the clinical recommendations in three RPL practical guidelines: the evidence-based guideline of the European Society of Human Reproduction and Embryology (ESHRE) of 2017, two consensus-based committee opinions of the American Society for Reproductive Medicine

(ASRM) considering definition and evaluation and treatment of RPL in 2013, and the evidence-based Royal College of Obstetricians and Gynaecologists (RCOG) guideline of 2011 (5-8).

MATERIALS AND METHODS

Three RPL guidelines of professional societies (ESHRE, ASRM and RCOG) were compared. The guidelines were assessed by three authors (AY, NV, MH). Their methodological similarities and differences were documented with the aim of comparing the definitions and clinical recommendations in the three guidelines. Rather than providing a full methodological assessment and comparison of the guidelines, we focused on the criteria most relevant to the aim of the current study. The criteria were selected from the AGREE II tool for assessment of quality and reporting of practice guidelines (9).

The following data were extracted from the three guidelines: definition of RPL, risk factors, diagnostic investigations and therapeutic options. For each aspect, the clinical recommendations were tabulated and an assessment made about whether there was agreement between the three guidelines. In cases of disagreement, we attempted to provide an explanation. The discrepancies and similarities between the guidelines, and the lack of evidence, are summarized later in this paper.

RESULTS

EXISTING GUIDELINES ON RPL

The included guidelines were developed by international (ESHRE, ASRM) and national (RCOG) professional organizations. The guidelines were published between 2011 and 2017. The methodological similarities and differences between the three guidelines are summarized in **Table 1**.

Two of the guidelines were supported by a literature search for evidence and a strict methodology for formulating recommendations (ESHRE, RCOG), while the other was mainly consensus-based. Most guideline development groups were composed of gynaecologists, with only one guideline (ESHRE) including experts from other domains (internal medicine, endocrinology, genetics, andrology and a patient representative). Conflicts of interest were documented in all three guidelines (ESHRE, ASRM and RCOG). All three

guidelines were externally reviewed by stakeholders before finalization and publication, but approaches to stakeholder involvement differed. All three societies are independent and are not externally financed.

Table 1 | Comparison of the most relevant AGREE II tools for the assessment of quality of the RPL practice guidelines

	ESHRE 2017	ASRM 2013	RCOG 2011
Multidisciplinary group (AGREE 4)	YES	NO	NO
Patient input (AGREE 5)	YES	NO	NO
Evidence search (AGREE 7)	YES	NO	YES
Methods for formulation of recommendations (AGREE 10)	YES	NO	YES
External review (AGREE 13)	YES	YES	YES
Tools for practice (AGREE 19)	YES[1]	NO	YES[1]
Resource implication (AGREE 20)	NO	NO	NO
Monitoring criteria (AGREE 21)	NO	NO	NO
COIs documented (AGREE 23)	YES	YES	YES

[1] These guidelines were supplemented with a patient version of the guidelines.

With regard to implementation, none of the guidelines included information on resource implications (i.e., which resources would be needed to implement the guidelines), or criteria for monitoring the uptake of the guidelines. Two of the guidelines (ESHRE, RCOG) were supplemented with a patient version, which can be considered a tool for clinical practice, contributing to the implementation of the guideline. One of the guidelines (ESHRE) is supplemented by a summary paper which may serve as a format for local protocols (5). None of the guidelines were supplemented by other tools such as decision aids, pathways or risk of pregnancy loss calculators.

DEFINITION OF RPL

There are four main components in the definition of RPL: defining pregnancy (biochemical, visualized, intrauterine), defining up to how many weeks the loss of pregnancy is considered a pregnancy loss, defining recurrence and deciding whether those recurring pregnancy losses have to be consecutive. The definitions of the guidelines are summarized in **Table 2**. The ASRM Committee Opinions advise confirming a pregnancy by ultrasonography or histopathology. The ESHRE guideline discusses the location of pregnancies and concludes that ectopic and molar pregnancies should not be included in the definition. The ESHRE and RCOG guidelines both mention that a miscarriage includes all pregnancy losses before the foetus reaches viability, i.e., up to 24 weeks of gestation. The ASRM guideline does not mention a time limit. The ESHRE and ASRM guidelines consider two pregnancy losses sufficient to meet the definition of RPL and consider diagnostic investigations. However, the ESHRE guideline states that not all guideline group members agreed to this definition. The RCOG guideline considers three pregnancy losses to meet the definition of recurrence. The last component of the definition is whether the recurring losses are obliged to be consecutive or not. The ESHRE guideline discusses the probability of including non-consecutive pregnancy losses, while the other guidelines only include consecutive pregnancy losses (**Table 2**).

Table 2 | Comparison of the elements of RPL definitions in three (inter)national guidelines

	ESHRE 2017	ASRM 2013	RCOG 2011
Pregnancy	Serum or urine hCG; ectopic and molar pregnancies not to be included in the definition	Clinical pregnancy documented by ultrasonography or histopathological examination	All pregnancy losses not further defined
Weeks of Gestation	Up to 24 weeks	Only mentions that majority is lost prior to 10th week	Up to 24 weeks
Recurrence	2	2	3
Consecutive	Consecutive or non- consecutive	Consecutive	Consecutive

INVESTIGATIONS

Investigations carried out in couples with RPL aim to detect underlying risk factors contributing to the losses, and can include assessment of lifestyle, genetic analysis, thrombophilia, immunologic and metabolic testing, assessment of uterine anatomy, and assessment of male factor contribution. The similarities and differences between the investigational advice in the guidelines are summarized in **Table 3**, and the differences highlighted. All three guidelines indicate an association between lifestyle (body mass index, smoking status, use of alcohol) and RPL, and two of the three guidelines (ESHRE and ASRM) mention that lifestyle should be investigated.

Genetic analyses can be divided into two main categories: parental genetic analysis and pregnancy tissue genetic analysis. Parental analysis can be performed to detect chromosomal abnormalities, which could increase the chances of a couple experiencing RPL. Analysis of the pregnancy tissue, however, may provide a reason for the particular pregnancy loss, but does not necessarily explain RPL in that couple. All three guidelines focus on the parental analysis, of which the ESHRE guideline determines that it should not be performed on a routine basis, but it could be carried out after individual assessment of risk. One guideline (RCOG) recommends starting with analysis of the pregnancy tissue on the third and subsequent consecutive pregnancy loss, and performing parental karyotyping only in case an unbalanced structural chromosome abnormality is found.

Thrombophilia can either be acquired or inherited. Acquired thrombophilia or antiphospholipid syndrome (APS) is an autoimmune syndrome caused by antibodies directed against phospholipids, leading to a hypercoagulable state. APS is characterized by thrombosis and/or pregnancy morbidity, including RPL. All guidelines recommend screening for anticardiolipin antibodies (ACA) and lupus anticoagulant (LAC). Furthermore, the ASRM guideline recommends screening for anti-β2-glycoprotein I, another antibody associated with APS. Regarding screening for inherited thrombophilia, the evidence is less clear, and screening is limited to cases with additional risk factors in all guidelines. The ESHRE does advise against measuring homocysteine levels because of inconsistent evidence regarding an association with RPL and possible pregnancy complications, and regarding the impact of several lifestyle factors on plasma homocysteine levels.

Table 3 | Comparison of investigations and therapeutic options of the RPL guidelines.

	ESHRE	ASRM	RCOG
Year of publication	2017	2012, 2013	2011
Risk factors			
	Age (female), anatomical factors, APS, embryonic factors, endocrine factors, genetic factors, lifestyle factors, preceding pregnancy losses	Anatomical factors, APS, embryonic factors, endocrine disorders, genetic factors, inherited thrombophilia, lifestyle factors	Anatomical factors, APS, embryonic factors, endocrine factors, genetic factors, immune factors, lifestyle factors, maternal age, preceding pregnancy losses, thrombophilia
Investigations			
General	Medical, obstetric and family history	Medical history related to RPL, lifestyle	-
Genetics	Parental karyotyping not on a routine base. Analysis of pregnancy tissue for explanatory purpose, using array-CGH	Parental karyotyping. Karyotyping of the products of conception may be useful in setting of on-going RPL therapy	Cytogenetic analysis on pregnancy tissue on third and subsequent consecutive pregnancy loss(es), parental karyotyping in case of unbalanced structural abnormality
Thrombophilia	No screening unless in context of research or in case of additional risk factors for thrombophilia	No screening unless in context of research or in case of additional risk factors for thrombophilia (FV Leiden, FII, Protein C, Protein S, and AT deficiencies)	FV Leiden, FII and protein S only in women with second trimester pregnancy loss

	ESHRE	ASRM	RCOG
Hyperhomocysteinemia	Not routinely recommended	-	-
Immunology/APS	ACA and LAC anti-β_2-glycoprotein I antibodies ((aβ2GPI) could be considered ANA could be considered	ACA, LAC and anti-β_2-glycoprotein I	ACA and LAC on two occasions 12 weeks apart
Endocrinology	Thyroid screening (TSH and TPO) followed up by T4 testing in case of abnormal screening results	Screening for thyroid (TSH) or prolactin abnormalities and test for HbA1c	-
Anatomy	3D ultrasound	Sonohysterogram, HSG and/or hysteroscopy	Pelvic ultrasound uterine anatomy. Suspected anomalies require further investigations using hysteroscopy, laparoscopy or 3D pelvic ultrasound
Male factor	Sperm DNA fragmentation can be considered	Sperm DNA fragmentation not recommended	-
Therapy			
Lifestyle	Health advice on diet, smoking and alcohol	-	-
Balanced chromosome abnormality	Genetic counselling, IVF + PGT optional	Genetic counselling if a structural genetic factor is identified, IVF + PGT optional	Genetic counselling, IVF + PGT optional
Thrombophilia	Insufficient evidence. Only in the context of research	-	Insufficient evidence

	ESHRE	ASRM	RCOG
Immunology/APS	Low dose aspirin (75-100 mg daily) starting before conception and a prophylactic dose heparin (unfractioned or low molecular weight starting at positive pregnancy test	Prophylactic dose of unfractioned heparin and low dose aspirin	Low dose aspirin plus heparin
Hyperhomocysteinemia	-	-	-
Endocrinology	Levothyroxine for hypothyroidism Vitamin D supplementation Bromocriptine treatment for hyperprolactinemia Other treatments not advised	Maternal endocrine disorders (thyroid dysfunction, diabetes and hyperprolactinemia) should be treated Other treatments not advised	Insufficient evidence
Anatomy	Insufficient evidence. Septum resection in context of trial	Consider surgical correction	Insufficient evidence
Unexplained RPL	No therapeutical interventions recommended	TLC. Emphasize chance for future successful pregnancy can exceed 50-60%	Supportive care of a dedicated early pregnancy assessment unit
Success rate subsequent pregnancy	Shown in a figure (17, 18)	Shown in a figure (18)	5% (17, 19)
Follow up	Supportive care	TLC. Psychological counselling and support	-

Several immunological aspects have been investigated and linked to RPL, including antinuclear antibodies (ANA), cytokine polymorphisms, human leukocyte antigen (HLA) typing and screening for HLA antibodies. ANA testing could be considered according to the ESHRE guideline, but there is no current treatment. Other immunological tests are not recommended in either of the guidelines. HLA typing, screening for HLA antibodies, and cytokine polymorphism testing are not recommended by the ESHRE or ASRM.

Metabolic testing in RPL is largely focused on thyroid function. Hypothyroidism is mentioned as a risk factor in every guideline. The ASRM and ESHRE guidelines recommend thyroid function screening; ESHRE suggests measurement of T4 in case of abnormal thyroid-stimulating hormone (TSH) or anti-thyroid peroxidase (anti-TPO) levels. The ASRM guideline advises testing the levels of HbA1c and prolactin for abnormalities.

Uterine examination is recommended by all guidelines. The ASRM guideline recommends examining the uterine cavity using hysterosonography (HSG), hysteroscopy or sonohysteroscopy. The RCOG suggests starting with pelvic ultrasound and applying HSG, hysteroscopy or sonohysteroscopy or 3D ultrasound in case of inconclusive findings, while ESHRE concludes that 3D ultrasound is the preferred technique to evaluate the uterus.

On male factors, the ESHRE guideline states that the assessment of sperm DNA fragmentation can be considered for explanatory purposes; the ASRM guideline, however, concludes that this assessment is not recommended.

THERAPY

Treatment options for RPL are limited and depend on the results of the investigations. The following interventions can be considered: lifestyle advice, IVF plus preimplantation genetic testing (PGT), aspirin and heparin, levothyroxine and surgery for uterine anomalies. Therapeutic advice from the guidelines is summarized in **Table 3** and the differences are discussed here.

The ESHRE guideline provides advice on lifestyle changes, regardless of the underlying cause: weight loss if BMI ≥25, healthy diet, cessation of smoking and reduction in alcohol consumption.

All guidelines recommend referring couples with detected chromosomal abnormalities to a clinical geneticist to offer a prognosis on future pregnancies. PGT can be a treatment for couples with parental genetic abnormalities, as by genetic testing of a few embryo cells on the presence of a previously diagnosed abnormality it allows for the selection of embryos without the genetic abnormality from the parents. RCOG discusses IVF plus PGT but recommends informing couples that their chances of a live birth through natural conception (50–70%) are higher than general live birth rates after IVF and PGT (about 30%). The ESHRE and the ASRM guidelines also discuss IVF plus PGT as an optional treatment. They conclude that data are limited, but that patients may be informed about the possibility, advantages and disadvantages of IVF plus PGT.

All guidelines advise on treatment for APS in women with RPL with low-dose aspirin plus heparin. ESHRE recommends starting low-dose aspirin (75–100 mg daily) before conception and a prophylactic dose of heparin (unfractionated or low molecular weight) once there is a positive pregnancy test. The other guidelines do not specify the order of aspirin and heparin.

The ASRM guideline only explores the screening of thyroid or prolactin abnormalities while the ESHRE and RCOG guidelines discuss other endocrine problems and treatment options. The ASRM guideline recommends treatment of endocrine disorders (such as diabetes, thyroid dysfunction when TSH levels are abnormal) in the context of RPL. ESHRE recommends treatment of hypothyroidism arising before or during early gestation with levothyroxine. ESHRE also suggests including general advice to consider vitamin D supplementation for all pregnant women, as concerns have been raised over the prevalence of vitamin D deficiency. Furthermore, the ESHRE guideline suggests bromocriptine treatment in case of hyperprolactinemia. The RCOG guideline concludes that there is insufficient evidence for the treatment of these causes. Other treatments such as progesterone supplementation, human chorionic gonadotrophin (HCG) supplementation and suppression of high LH is not advised.

In case of an anatomical uterus abnormality, the ASRM guideline suggests considering resection. However, the RCOG concludes that there is insufficient evidence so far for this treatment. Likewise, the ESHRE guideline concludes that the effect of septum resection should be evaluated in randomized controlled trials and that other uterine reconstructions are not recommended. The TRUST trial (Dutch trial number: NTR 1676) is currently investigating whether hysteroscopic septum resection improves the reproductive outcome in women with a septate uterus (10). Regarding acquired intrauterine malformations, the ESHRE and ASRM conclude that there is insufficient evidence for the removal of fibroids or adhesions (Asherman syndrome).

All guidelines aim to improve success in subsequent pregnancies. Emphasis on the relatively high chances of success focus points of the ASRM Committee Opinions. The RCOG guideline gives information on the psychological aspects and provides patients with support via a specialized care unit, and the ESHRE guideline recommends different interventions based on the current available evidence.

Supportive care is suggested to comfort patients in the ASRM and the ESHRE guideline.

DISCUSSION

This paper describes a methodology assessment (AGREE II), summarizes and compares the recommended definitions, risk factors, investigations and therapies of three guidelines considering RPL. It is clear that discrepancies exist between the three guidelines, which could explain variation in practice. Also notable is that the ESHRE and RCOG guidelines have a similar format, while the ASRM has the style of a review, but discusses the same subjects and makes evidence-based recommendations on those subjects.

Guidelines are living documents, subjected to updates based on recent trial results, and they should inspire clinicians to base their clinical practice on the most recent available data.

To ensure couples with RPL all over the world receive comparable and preferably evidence-based diagnostic investigations and treatment options, only one internationally accepted guideline is in fact needed. When evidence shows what diagnostic methods and what treatments are

beneficial, it is unnecessary to have different guidelines in all countries. Of course, not all the recommendations are applicable to all populations worldwide, but if countries are similar in terms of medical services and populations, guidelines could be unified. The ESHRE guideline, for example, is available for official consideration by any professional society in obstetrics and gynaecology.

One major hurdle in the universal application of one guideline is the inconsistency of the definition criteria of RPL, which appears to be more a discussion based on opinions rather than evidence. If not, internationally consistent it will inherently lead to discrepancies in therapy of RPL. The definition will also have significant resource implications, as it will define when to start performing investigations.

Jaslow et al. (2010) showed that there is no difference in the prevalence of abnormal test results in women with different numbers of RPL. Furthermore, the risk of miscarriage after two consecutive miscarriages is clinically similar to the risk of recurrence among women with three or more consecutive pregnancy losses (11). There is evidence that the risk of APS is not associated with the number of pregnancy losses or with pregnancy losses being consecutive (12). Carrier status of a balanced chromosomal abnormality does not differ between couples with two or three consecutive losses versus woman with two or three non-consecutive losses (13). The ASRM document mentions that for epidemiological studies, a threshold of three or more losses should ideally be used. For the sake of this emotionally stressful situation and based on studies showing that there seems to be no differences in finding a cause between two or three losses, women with two losses should be offered evaluation. Egerup et al. argued that only consecutive pregnancy losses should count in the definition of RPL, showing that a birth in women with secondary RPL eradicates the negative prognostic impact of previous pregnancy losses (14). The ESHRE guideline includes non-consecutive losses in its definition. Furthermore, Kolte et al. states that non-visualized pregnancies should also be included in the definition of RPL, as does the ESHRE guideline (15). This study showed that non-visualized pregnancies are of prognostic importance, although only assessed in women with idiopathic RPL.

Risk factors and investigations are generally similar between the compared guidelines. Discrepancies between the recommendations in the guidelines can be explained partly by their methods of development and the time of their publication, and the lack of strong evidence on some clinical aspects of RPL.

In general, guidelines are believed to stay up to date for 4 years. The three guidelines were published at different time points, ranging from 2011 to 2017, and this explains some of the differences. For example, homocysteine levels and maternal thrombophilia are no longer considered to be associated with RPL. The most recent guideline (ESHRE) therefore no longer advises performing these tests. Another example is the focus toward thyroid function testing in the more recent guidelines (ESHRE, ASRM). Although the format of the ASRM guideline differs from the ESHRE and RCOG, being written in the form of a review, we could not detect significant differences, as the ASRM also reviewed the available literature, as did the ESHRE and RCOG guidelines.

The absence of sufficient evidence could explain the differences regarding the investigation and treatment of uterine malformations, and most of the endocrinological and immunological tests and treatments. For testing hereditary thrombophilic factors and treatment of hereditary thrombophilia, all guidelines agree on the lack of evidence. Currently, the ALIFE II study is investigating whether antithrombotic treatment in patients with RPL would result in higher live birth rates in comparison with placebo (16). The recent ESHRE guideline emphasizes that many recommendations in this field are still based on a low level of evidence also documented in a recent publication (5). The guideline was supplemented with a research agenda outlining aspects of RPL for which studies should be performed to reduce uncertainty, and improve care of couples in the near future.

Finally, one significant difference between the guidelines cannot be clearly attributed to method of development or lack of evidence, which is the difference in genetic investigations between the RCOG and the other guidelines. The RCOG advises selective parental karyotyping when an unbalanced chromosome abnormality is identified in the pregnancy tissue, because of the high costs and the relatively low incidence of unbalanced

chromosome abnormalities. The other guidelines advise parental karyotyping according to the risk table, and do not routinely investigate the pregnancy tissue.

In conclusion, the current paper describes the similarities and differences between clinical recommendations provided in three RPL guidelines, and attempts to explain some of the differences based on the time and method of development, and on the lack of supporting evidence. As a clinician it is important to realize that there are differences in the guidelines considering the treatment of RPL. Nowadays there is an increasing range of tests and therapies offered to women attending recurrent miscarriage clinics. Couples that suffer from RPL should be informed about the possible investigations and treatment options, and whether those are evidence-based.

We advise following the most recent guidelines, being aware of discrepancies and only making use of evidence-based therapies. To answer the questions for which evidence is lacking and improve the future care for RPL couples, new trials could be set up and patients could be asked to participate in an ongoing randomized controlled trial.

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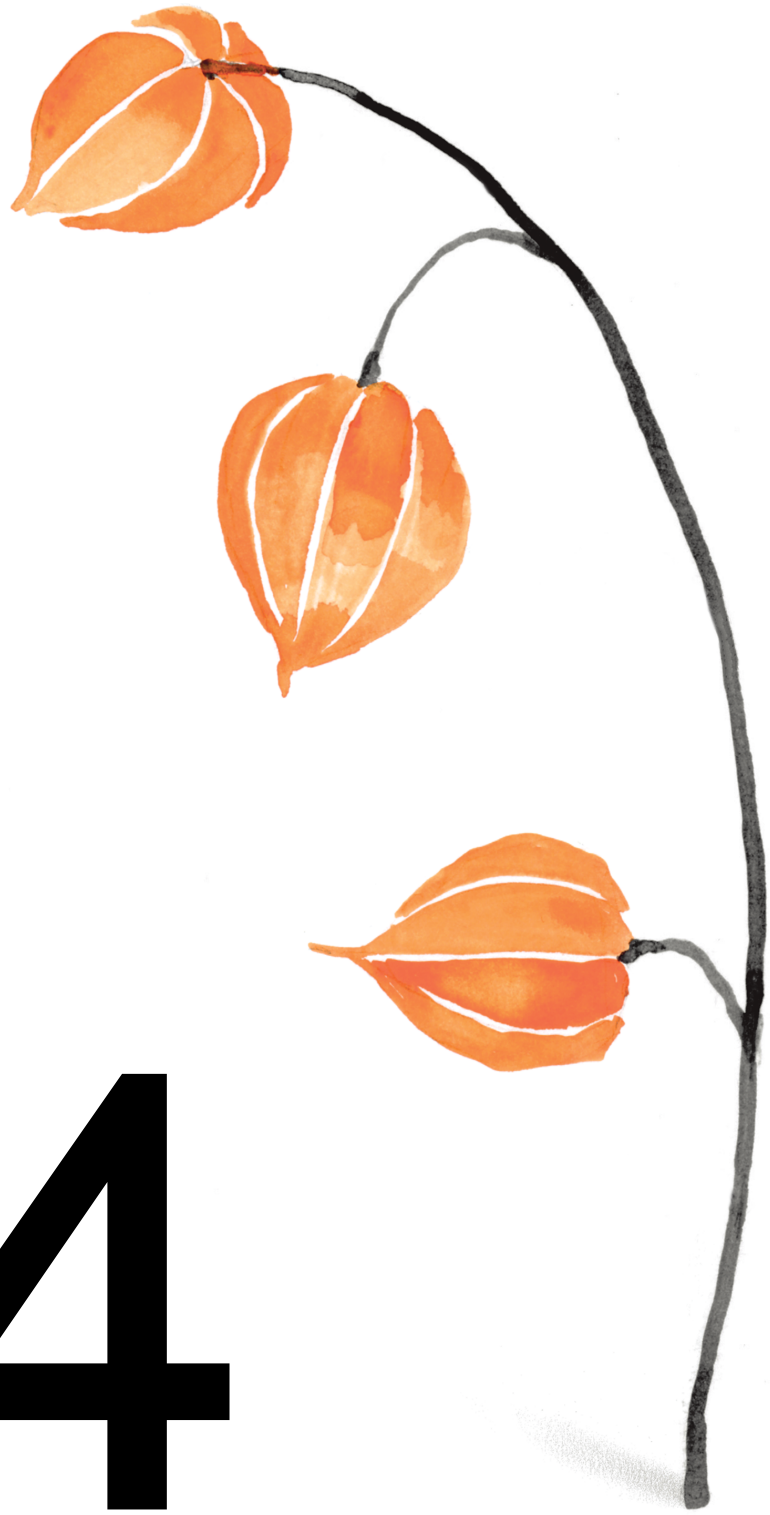

4

CHAPTER 4

CLINICAL PRACTICE VERSUS EVIDENCE-BASED GUIDELINE IN RECURRENT PREGNANCY LOSS CARE

A. Youssef, E.E.L.O. Lashley, N. Vermeulen, M.L.P. van der Hoorn

Based on: identifying discrepancies between clinical practice and evidence-based guideline in recurrent pregnancy loss care, a tool for clinical guideline implementation.

BMC Pregnancy and Childbirth, 2023

ABSTRACT

BACKGROUND

Practice variation in recurrent pregnancy loss (RPL) care is common. International guidelines vary in their recommendations for the management of RPL couples, which could lead to an increase of cross border reproductive care. Currently, the Dutch RPL guideline is being adapted from the European Society for Human Reproduction and Embryology (ESHRE) guideline. We aim to identify discrepancies between RPL guidelines and RPL practice. These discrepancies could be considered in the development of a new guideline and implementation strategies to promote adherence to new recommendations.

METHODS

A nationwide survey on the management of RPL patients was conducted across all 107 hospital-based obstetrics and gynaecology practices in the Netherlands. The survey was sent via the Dutch Society for Obstetricians and Gynaecologists to all affiliated clinicians. The questionnaire consisted of 36 questions divided in four sections: clinician's demographics, RPL definition, investigations and therapy. The data were compared to the recommendations given by the Dutch national guideline and the most recent guideline of the ESHRE.

RESULTS

All hospital-based practices (100%; n=107) filled in the online questionnaire. The majority of respondents defined RPL similarly, as two or more pregnancy losses (87.4%), not obligatory consecutive (93.1%). More than half of respondents routinely perform thrombophilia screening (58%), although not advised by the ESHRE, while thyroid function (57%), thyroid auto-immunity (27%) and β2-glycoprotein antibodies (42%) in the context of antiphospholipid syndrome (APS) are recommended but investigated less often. Regarding parental karyotyping, 20% of respondents stated they always perform parental karyotyping, without prior risk assessment. because of RPL. Treatment for hereditary thrombophilia was frequently (43.8% (n=137)) prescribed although not recommended. And finally, a considerable part (12-16%) of respondents prescribe medication in case of unexplained RPL.

CONCLUSION

While many clinicians perform investigations recommended by the ESHRE, there is a considerable variation of RPL practice in the Netherlands. We identified discrepancies between RPL guidelines and RPL practice, providing possibilities to focus on multifaceted implementation strategies, such as educational intervention, local consensus processes and auditing and feedback. This will improve the quality of care provided to RPL patients and may diminish the necessity felt by patients to turn to multiple opinions or cross border reproductive care.

BACKGROUND

Recurrent Pregnancy Loss (RPL) is defined as the loss of two pregnancies before 24th week gestation (1). Despite extensive investigations, RPL remains unexplained in more than half of couples (1). This affects couples' psychological health and their quality of life. Therefore, an important role is preserved for the managing physician, to support and guide these couples through the many investigations and treatments.

In previous studies we have shown that although national and international guidelines exist, protocols still vary (1-6). This high level of practice variation might lead to patients seeking care in various (inter)national centres, and be offered more extensive investigations and treatments.

In the Netherlands, the RPL guideline was published in 2007 by the Dutch Society for Obstetricians and Gynaecologists (NVOG) (7). New evidence has been published since regarding definition, investigations and treatments. Therefore, the guideline is in need of revision, which is currently conducted based on the ESHRE guideline (1). This ESHRE guideline is developed based on up-to-date evidence with strict guideline development methodology (8). To make sure that clinicians will adhere to recommended investigations and treatments, different strategies to implement evidence-based guidelines are suggested (9). One of the suggested strategies is to audit the current performance of health care providers. In this study we therefore conducted a nationwide cross-sectional survey on current clinical management of RPL patients across all 107 obstetric- and gynaecology practices in the Netherlands, and compared the results with the most recent evidence-based guideline developed by the ESHRE.

METHODS

In this cross-sectional survey study, an online questionnaire (Castor EDC) was sent to all 107 obstetric- and gynaecology practices in the Netherlands in November 2020, with the Leiden University Medical Centre as primary research centre. Eight of these hospitals were university hospitals, 62 teaching and 37 non-teaching hospitals. The questionnaire consisted of 36 questions in total which were divided in four sections: clinician's demographics, RPL definition, investigations and therapy (Appendix 1). The survey was conducted over a three months period until all practices had

completed the survey at least once (November 2020 until January 2021). The survey was sent via the Dutch Society for Obstetricians and Gynaecologists (to which all obstetrics and gynaecology practices are adjoined) to all affiliated clinicians. This includes residents, fertility doctors and medical specialists; all respondents participated the same survey. We aimed to obtain at least one response from 75% of all hospitals. After a second invitation to hospitals that had not yet responded, lead clinicians were contacted by mail.

Data is presented as percentages of respondents that indicated the specific answer choice over the total of respondents that answered the question. Between parentheses the number of replies to the corresponding question is given. Data were compared to the Dutch national (7) and the ESHRE (1) recommendations, as currently the ESHRE guideline is being adapted for a new RPL guideline in the Netherlands. Furthermore, data of university hospitals were compared to non-university hospitals using the Chi-squared test, with statistical significance when $p < 0.05$.

RESULTS

RESPONDENT DEMOGRAPHICS

All hospital-based practices (100%; n=107) filled in the online questionnaire. A total of 446 entries were registered in the online questionnaire database. Of all entries, 315 were returned with 100% completion and 45 questionnaires were returned with at least 50% completion. The participants were primarily gynaecologists (71.7%; n=320/446) or obstetrics and gynaecology residents (22.4%; n=100/446), the remaining participants (5.8%; n=26/446) were 24 fertility doctors, one nurse and one medicine student. Half of all questionnaires were returned from non-university teaching hospitals (50.7%; n=226/446), 23.1% (n=103/446) by non-teaching hospitals and 21.1% (n=94/446) by university hospitals. In addition, 20 entries were returned from private clinics and three remained unknown.

DEFINITION

The majority of respondents defined RPL as two or more pregnancy losses (87.4%; n=346/394), not necessarily consecutive (93.1%; n=367/394). Ectopic pregnancies (14.8%; n=58/393), pregnancy of unknown location

(31.3%; n=123/393) and molar pregnancies (12.2%; n=48/393) were included in the definition of RPL by a minority of respondents and biochemical pregnancies were included in the definition by 45.0% (n=177/393) of respondents. Both spontaneous and assisted reproductive technology (ART) pregnancies were counted in RPL obstetric history by 93.8% of the respondents (n=366/390) (Table 1). The Dutch guideline defines RPL as two or more pregnancy losses before 20 weeks of gestation, excluding ectopic, molar and biochemical pregnancies. The ESHRE guideline includes biochemical pregnancies and pregnancies of unknown location in the definition, as well as ART pregnancies.

Table 1 | RPL definition components used by respondents (in %) with recommendations of dutch guideline and ESHRE guideline for each component

	Number of respondents	Respondents (%)	Dutch guideline	ESHRE guideline
Number				
2	346/396	87	√	√
3	47/396	12		
More than 3	3/396	1		
Consecutiveness				
Consecutive	26/394	7		
Non-consecutive	367/394	93	√	√
Pregnancy type included				
Intra-uterine pregnancy	383/393	98	√	√
Extra-uterine pregnancy	58/393	15		
Molar	48/393	12		
Biochemical	177/393	45		√
PUL	123/393	31		√
Origin			*	
Spontaneous	24/390	6		
ART and spontaneous	366/390	94		√
Obstetric history and relationship				*
All pregnancies	26/388	58	√	
Only current relationship	162/388	42	√	

PUL: pregnancy of unknown location; ART assisted reproductive technology
√ Recommended
* Not indicated

INVESTIGATIONS

The results of the investigations considered in this questionnaire are listed in Table 2, including the recommendations of both the Dutch national guideline and ESHRE. Most respondents initiate investigations after two pregnancy losses (87.3%; n=324/371), and start with obtaining information on general aspects such as body weight and length (93.3%; n=348/373), and lifestyle (90.2%; n=343/373).

According to the questionnaire, approximately 70% of respondents initiate parental karyotyping after risk assessment based upon the maternal age at second miscarriage, number of preceding miscarriages and history of miscarriages in either the siblings or in the parents (10). Twenty percent responded that they always perform parental karyotyping. Genetic testing on pregnancy tissue after miscarriages is performed by 2.2% (n=8/363) of respondents. Both guidelines recommend karyotyping only on indication. The ESHRE recommends pregnancy tissue testing only for explanatory purposes.

Almost all participants offer APS investigations (98.9%; n=346/350); lupus anticoagulant and anticardiolipin antibodies (ACA) are generally performed by most participants (see Table 2), anti-β2-glycoprotein antibodies testing is performed by less than half of respondents (IgM testing: 36.9%; n=129/350 and IgG testing: 42.3%; n=148/350). Both guidelines recommend APS testing.

Approximately half of the participants perform Thyroid Stimulating Hormone (TSH) testing (56.5%; n=195/345), and 26.7% performs Thyroid Peroxidase (TPO) antibodies testing (n=92/345). Twenty-four percent of the participants indicate that they do not perform any endocrine testing (n=107/345). The Dutch guideline does not recommend thyroid screening, while the ESHRE does recommend both function testing and auto-immunity thyroid testing.

Two-dimensional ultrasound was the most performed investigations according to this questionnaire (76.7%; n=266/374). Three-dimensional ultrasound was preferred only by 8.9% of participants (n=31/374). Three-dimensional ultrasound is the preferred technique as mentioned by the ESHRE guideline. The Dutch guideline does not mention testing for uterine malformations.

Regarding the male partner, respondents usually acquired male lifestyle information (60.1%; n=199/331). A minority of responders also performed investigations, such as sperm DNA fragmentation (3.0%; n=10/331) or semen analysis (1.2%; n=4/331). This investigation is recommended by the ESHRE only for explanatory purposes. The Dutch guideline does not mention testing for male factors.

An overview of the adherence to recommended investigations of the ESHRE and Dutch national guideline is provided in Figure 1.

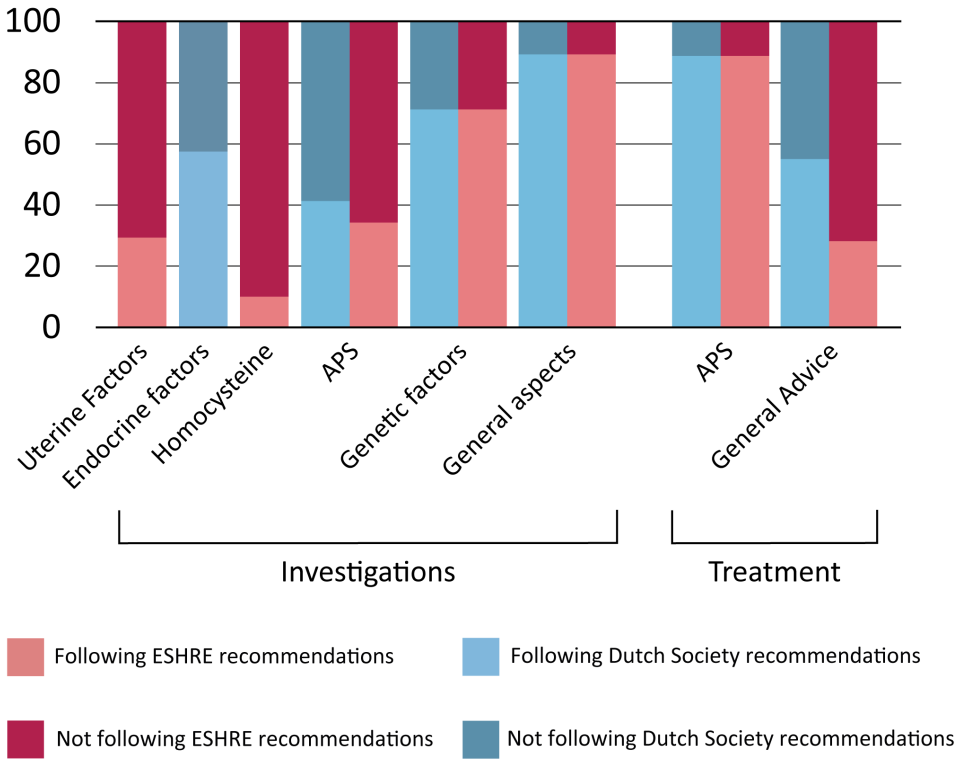

Figure 1. Representation of the percentage of respondents (Y-axis) that perform RPL care in line with the recommendations of the Dutch Society for Obstetricians and Gynaecologists and the ESHRE for investigations (left part on the X-axis) and treatment (right part on the X-axis). Categories on the X-axis contain all investigations and treatments that are recommended to be performed (non-recommended investigations and treatments are not included in percentages portrayed). General aspects include BMI, lifestyle and blood pressure. General advice refers to advice for smoking cessation, alcohol cessation, weight loss, and folic acid and vitamin D supplementation.

NON-RECOMMENDED INVESTIGATIONS

The ESHRE and the Dutch guideline suggests not to screen for hereditary thrombophilia and/or hyperhomocysteinemia, unless in the context of research or in the presence of additional risk factors. Up to 58% (n=206/358) of respondents indicated that they perform some form of thrombophilia investigations, and more than a quarter (29.1%; n=104/358) indicated that they only perform hereditary thrombophilia investigations in case of additional risk factors.

Table 2 | Investigations performed by respondents with recommendations of the Dutch and ESHRE guideline.

Investigations	Number of respondents	Respondents (%)	Dutch guideline	ESHRE guideline
General Aspects			√	√
BMI	348/373	93	Yes	Yes
Lifestyle	343/373	92	Yes	Yes
Blood pressure	93/373	25	*	*
Genetic factors				
Female karyotype†	262/363	72	Ø	Ø
Male karyotype†	260/363	72	x	Ø
Pregnancy tissue array	8/363	2		Ø
Antiphospholipid syndrome			√	√
ACA IgG	318/350	91	Yes	Yes
ACA IgM	294/350	84	Yes	Yes
Lupus Anticoagulant	321/350	92	Yes	Yes
Anti-β2-glycoprotein IgG	148/350	42	*	Yes
Anti-β2-glycoprotein IgM	129/350	37	*	Yes
Endocrine factors				
TSH	195/345	57	x	√
TPO antibodies	92/345	27	*	√
T4 and/or T3	82/345	24	x	x
Progesterone	6/345	2	x	x
LH/FSH	7/345	2	x	x
Glucose	51/345	15	x	x
HbA1c	29/345	8	x	x
Uterine factors			*	√
2D ultrasound	266/347	77		
3D ultrasound	31/347	9		∫
HSG	11/347	3		
Hysteroscopy	55/347	16		
SIS	71/347	21		
MRI	4/347	1		
Ongoing pregnancy				*
hCG	7/333	2	*	
Progesterone	5/333	2	*	

Pregnancy tissue	7/333	2	x	
karyotype	5/333	2	*	
Pregnancy tissue array	270/333	81	Yes	
Ultrasound	2/333	1	*	
HbA1c	5/333	2	*	
Glucose				
Thrombophilia			Ø	x
Antithrombin – III	105/358	29	Yes	
APC-resistance	83/358	23	Yes	
APTT	37/358	10	*	
Factor II mutation	80/358	22	Yes	
Factor V Leiden	105/358	29	Yes	
Factor VIII	41/358	12	Yes	
Factor X	16/358	5	*	
Protein C	120/358	34	Yes	
Protein S	127/358	36	Yes	
Fibrinogen	18/358	5	*	
INR	9/358	3	*	
Thrombin time	20/358	6	*	
Homocysteine	206/358	58	Yes	
Plasminogen	4/358	1	*	
Infections			*	x
CMV	9/342	3		
Chlamydia	17/342	5		
Gonorrhoea	12/342	4		
Immunological factors			*	
NK-cell plasma level	2/342	1		x
NK-cell level in EB	1/342	0		x
HLA typing and sharing	2/342	1		x
HLA antibodies	2/342	1		x
ANA antibodies	13/342	4		=

√ Recommended; * Not indicated; Ø On indication; x Not recommended; ∫ Preferred technique; = explanatory purpose

† Number indicated regards percentage of respondents that perform karyotyping according to individual risk assessment table.

BMI: body mass index; ACA: anticardiolipin antibodies; TSH: Thyroid Stimulating Hormone; TPO: thyroid peroxidase; HSG: hysterosalpingography; SIS: saline infusion sonohysterography; HbA1c: glycated haemoglobin; CMV: cytomegalovirus; NK: natural killer; EB: endometrial biopsy; HLA: human leukocyte antibodies; ANA: antinuclear antibodies

TREATMENT

The ESHRE advises to discuss health behaviour modifications such as cessation of smoking, striving for a normal range BMI, and limiting alcohol consumption. Smoking cessation was the most given general advice (95.4%; n=310/325), followed by folic acid supplementation (89.2%; n=290/325) and weight loss (78.2%; n=254/325).

Respondents usually treated APS (59.7%; n=190/318) in a next pregnancy, or referred patients for treatment to an internal medicine specialist or haematologist (28.9%; n=92/318). The treatment consisted of Low Molecular Weight Heparin (LMWH) and aspirin, the order and starting time of these medications differed between respondents (such as starting from conception or from foetal heartbeat on ultrasound). The ESHRE recommends in treating patients diagnosed with APS in a next pregnancy with aspirin preconceptionally and LMWH in prophylactic dose starting at the day of a positive pregnancy test.

Treatment of hereditary thrombophilia was given by 43.8% (n=137/313) of respondents and usually consisted of LMWH and/or aspirin. A third of respondents (35.5%; n=111/313) referred patients for such treatment. Uterine septum correction was performed by 12.2% of respondents (n=37/304). Patients with TPO antibodies were treated by half of respondents (51.5%; n=158/307). Thyroid function was followed up during next pregnancy by 43.0% of the respondents (n=132/307). The ESHRE does not recommend treatment for patients with hereditary thrombophilia or uterine septum, and states there is insufficient evidence to treat euthyroid women with thyroid antibodies

When asked whether any treatments were given to unexplained RPL couples, 16.8% (n=51/303) provided progesterone supplementation and 12.5% (n=38/303) provided aspirin treatment in next pregnancy. A small percentage of respondents also prescribed other experimental treatments for patients with unexplained RPL (Table 3)

An overview of the adherence to recommended treatments of the ESHRE and Dutch guideline is given in Figure 1.

Table 3 | Treatments performed by respondents (%) with recommendations of the Dutch and ESHRE guideline.

Investigations	Number of respondents	Respondents (%)	Dutch guideline	ESHRE guideline
General advice			√	√
Smoking cessation	310/325	95	Yes	Yes
Alcohol cessation	217/325	67	Yes	Yes
Weight loss	254/325	78	Yes	Yes
Folic acid	290/325	89	*	Yes[+]
Vitamin D	142/325	44	No	Yes[+]
Genetic factors			√	√
Genetic counselling	184/318	58		
PGT	76/318	24		
Uterine factors			x	x
Septum resection	37/304	12		
Endocrine antibodies (TPO)	26/307	9	x	x
Antiphospholipid syndrome	190/318	60	√	√
Thrombophilia	137/313	44	x	x
Unexplained RPL			x	x
Progesterone	51/303	17		
hMG	1/303	0		
IVF	2/303	1		
HCG	3/303	1		
Thyroxine	4/303	1		
Corticosteroids	3/303	1		
IVIG	0/303	0		
Intralipids	0/303	0		
LMWH	12/303	4		
Aspirin	38/303	13		
Donor insemination	0/303	0		
Oocyte donation	0/303	0		
Endometrium scratching	1/303	0		
Cross border care referral	23/303	8		

√ Recommended; * Not indicated; x Not recommended;

[+] Not recommended for improving live birth in RPL, but for general health purposes

ESHRE GUIDELINE

Half of the respondents had knowledge of the existence of the ESHRE guideline (49.3%; n=149/302), and 38.4% (n=116/302) indicated that they have implemented this guideline. University hospitals were more familiar with the ESHRE guideline (61.3% (n=38/62) vs 46.3% (n=111/240); p=0.035) and used this in daily practice (53.2% (n=33/62) vs 34.6% (n=83/240); p=0.007). Although a minority already uses the ESHRE

guideline, three-quarter of respondents indicated their approval of the Dutch society of gynaecology and obstetrics adapting the ESHRE guideline in Dutch practice (74.1%; n=223/301).

UNIVERSITY VERSUS NON-UNIVERSITY HOSPITALS

Comparison between university and non-university hospitals showed a statistically significant difference in two questions regarding definition, namely including biochemical pregnancies (57.0% (n=49/86) vs 41.7% (n=128/307); p=0.012) and the inclusion of couple specific pregnancy losses (27.4% (n=23/84) vs 45.7% (n=139/304); p=0.003).

Anti-β2-glycoprotein IgG and IgM were investigated more often in university hospitals compared to non-university hospitals (IgG 56.2% (n=41/73) vs 38.6% (n=107/277) and IgM 49.3% (n=36/73) vs 33.6% (n=93/277) (p=0.007 and p=0.013).

Both TSH and TPO-antibodies investigations were more often performed in university hospitals (TSH 69% (n=49/71) vs 53.3% (n=146/274); p=0.017 and TPO-antibodies 36.6% (n=26/71) vs 24.1% (n=65/274); p=0.033). University hospitals also used 3D ultrasound for the investigation of uterine anomalies more often (31.9% (n=23/72) vs 2.9% (n=8/275); p<0.001).

Homocysteine screening was performed almost twice as often in non-university hospitals (35.2% (n=25/71) vs 63.1% (n=181/287); p<0.001). Furthermore, thrombophilia screening in the context of scientific studies was performed more often in university hospitals (16.9% (n=12/71) vs 3.8% (n=11/287); p<0.001)

DISCUSSION

In this cross-sectional survey study, we audited the performance of healthcare providers on RPL guideline adherence. We observed that Dutch clinicians generally adhere to advised investigations and interventions (Figure 1), though there is room for improvement.

In defining RPL, the ESHRE includes biochemical- and resolved pregnancies of unknown location. In our survey, <50% of respondents followed this definition (Table 1), This may lead to an underestimation of RPL and exclusion of patients for further examination and treatment (11).

Considering discrepancies in investigations, we showed that 58% of respondents routinely perform thrombophilia screening, though not advised by the ESHRE (1). The wide application of this screening can be explained by the long inclusion period of the ALIFE-2 study (12). In the presence of a thrombophilic factor, clinicians may be tempted to start treatment, explaining the proportion of clinicians indicating treatment of hereditary thrombophilia in RPL couples (Table 3). Regarding parental karyotyping, 20% of respondents stated they always perform parental karyotyping, regardless of the risk assessment. Both the Dutch- as the ESHRE guideline recommends risk assessment prior to parental karyotyping, though this risk assessment is different amongst the guidelines, resulting in different number of karyotype testing. Regarding treatment discrepancies, we showed that a substantial portion of respondents advised progesterone or aspirin in patients with unexplained RPL. The ESHRE does not recommend treatment for patients with unexplained RPL, as no significant benefit was shown (13, 14). A recent trial however showed a possible effect for the use of progesterone in women with ≥3 pregnancy losses presenting with early bleeding in a next pregnancy (15). This could have implications for future recommendations on progesterone administration in patients with RPL.

To update the national RPL guideline and increase adherence to evidence-based RPL practice, adaptation of the ESHRE guideline is currently conducted in the Netherlands (1, 7). While the ESHRE and the Dutch guidelines are similar in some respects, they also contain different recommendations either based on data published after finalization of the Dutch guideline, or differences in expert opinion in areas lacking studies. Implementation of the ESHRE guideline could therefore be hampered. Barriers identified in a European questionnaire (16) were the lack of a Dutch translation and the fact that guidelines are long and difficult to understand. Information on clinical practice with regards to these aspects is helpful to identify discrepancies for better implementation of future guidelines.

Recently, Manning et al. have performed a comparable study in the UK and showed equivalent results regarding practice variation (17). They explained that in many practices dedicated RPL specialists were absent, who can strive for a consistent management of RPL couples. Our results support a similar conclusion based on practice variation between university and non-

university clinics. Indeed, university hospitals show more often a definition and policy congruent with the current ESHRE guideline.

We believe that a multifaceted implementation strategy could help improve guideline adherence, and thus evidence-based practice. It also could reduce unnecessary medical costs (17). This strategy implies educational intervention, such as disseminating of summary of the recommendations or the development of a web-based tool. In addition, local consensus processes for care that lack scientific evidence could help minimizing practice variation. And finally, auditing of healthcare workers' performance and feedback could act as an incentive to improve a clinician's management of RPL patients. Multifaceted implementation strategies are however not widely present regarding guideline implementation.

A major strength of our study is that we achieved 100% response rate, as all hospital-based practices have participated in this survey. This resulted in the elimination of sampling bias. Our study confirms previous findings of variation in practice and limited adherence to national guidelines (2, 6, 18, 19). We showed that ESHRE is rightfully concerned about the implementation of the RPL guideline (20).

A limitation of this study is that it was not possible to elucidate why clinicians persist or refrain from certain investigations and therapeutic options. This could have demonstrated a rationale for the demonstrated practice variation. Furthermore, survey studies are susceptible to desirability bias. It was not possible to measure whether participants gave any desirable answers, knowing guideline recommendations but performing otherwise in daily practice.

CONCLUSION

While many clinicians perform investigations recommended by the ESHRE, we also identified considerable discrepancies. Clinicians tend to rely more on guidelines published by national societies. To limit practice variation and thereby delivering care up to maximum standards, it is necessary that efforts of both overarching societies such as the ESHRE and local societies are collaborating in implementation of up-to-date guidelines. Using implementation strategies to improve guideline adherence will ultimately lead to better care delivered to RPL patients.

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5

CHAPTER 5

EVALUATION OF AN IMPLEMENTATION STRATEGY TO IMPROVE GUIDELINE ADHERENCE IN COUPLES WITH RECURRENT PREGNANCY LOSS

E. van den Boogaard, A. Youssef[+], E. Rijgersberg, M.L.P. van der Hoorn, H.R. Verhoeve, J.A.M. Kremer, M. Goddijn, R.P.M.G. Hermens

[+] Shared first author

Submitted

ABSTRACT

INTRODUCTION

Clinical management of couples with recurrent pregnancy loss (RPL) is often not in accordance with guideline recommendations, resulting in costly and ineffective management of couples with RPL. It is known from guideline implementation research that dissemination of new guidelines alone is not enough to achieve proper guideline adherence and that robust implementation efforts are necessary. Unfortunately, no gold standard exists for successful implementation of new evidence. The objective of this study was therefore to test a multi-faceted implementation strategy on its capability to improve guideline adherence in couples with RPL.

MATERIALS AND METHODS

A cohort study was performed in nine Dutch hospitals within a 12-month period before and a six-month period after the introduction of the strategy. A systematically developed strategy was introduced in the Obstetrics and Gynaecology departments of four hospitals in the Netherlands. Guideline adherence in women with RPL was measured before and after introduction of the implementation strategy. Indicators covered diagnostics, therapy and counselling. Multilevel analyses were performed to compare the change in guideline adherence after the introduction of the strategy. A cost-effectiveness analysis was performed from a health care perspective.

RESULTS

356 women were included before and 243 after introduction of the strategy. Adherence was significantly higher on most indicators on diagnostics and counselling. The highest increase was measured for selective screening for thrombophilia (+37%, Odd Ratio (OR); 5.2, 95% Confidence Interval (CI) 3.6-7.6). The use of the specified medical chart file, patient questionnaire, pocket card and electronic decision program were related to higher adherence. Health care costs in the four participating centres were reduced with 206,916 euros annually.

CONCLUSION

Adherence to the guideline on RPL improved after introduction of the implementation strategy, the strategy was feasible and effective and costs were reduced. This implementation strategy can widely be introduced in clinical practice for RPL, and may serve as an example for future implementation strategies in other areas within obstetrics and gynaecology.

INTRODUCTION

Clinical management of couples with recurrent pregnancy loss (RPL) is often not in accordance with clinical evidence as summarized in guidelines (1-4). Under as well as over diagnostics are observed, resulting in an overall costly and ineffective management of couples with RPL (5). It is known from guideline implementation research that dissemination of new guidelines alone is not enough to achieve proper guideline adherence and that robust implementation efforts are necessary (6, 7).

Although intended to be revised regularly, revision of complete guidelines is a time-consuming process. At the time guideline adherence was measured, the RPL guideline from the Dutch Society of Obstetrics and Gynaecology (NVOG) was just published in the Netherland (3) and adopted by the National Guideline Clearinghouse (2009) (1). A recent study compared guidelines from the European Society of Human Reproduction and Embryology (ESHRE) (8), the British Royal College Obstetrics and Gynaecology (9) and a committee opinion of the American Society for Reproductive Medicine (ASRM 2013) (10, 11), showing both similarities and differences in RPL practice (12). These guidelines were partly in agreement with the Dutch guideline. Currently, an updated version of the ESHRE guideline is being expected, as well as a new RCOG guideline.

Unfortunately, no gold standard exists for successful implementation of new evidence. The most frequently studied interventions include audit and feedback on current quality of care and the dissemination of educational materials, with varying efficacy (13, 14). The effects of patient centred strategies in implementation in reproductive medicine have been explored, and showed varying degrees of success on implementation of guidelines (15-17). In other words, it is not obvious which implementation strategy has to be applied to improve guideline adherence and thereby quality of care in couples with RPL, but strategies tailored to existing barriers and facilitators have the best potential to gain effect in improving guideline adherence (15, 18). The main facilitators for guideline adherence in RPL are lower maternal age, adverse obstetric history, and visiting a doctor knowledgeable in RPL (19). The most important barriers are the guideline being too complicated to be used in the consultancy room, lack of up-to-date patient information and patients' lack of detailed knowledge about

family history. Based on these data, we developed a multi-faceted implementation strategy. The strategy consists of various elements for doctors to tackle complexity and elements for patients to improve information supply and knowledge.

The objective of our study was to test the multi-faceted implementation strategy on its capability to improve guideline adherence in couples with RPL.

MATERIALS AND METHODS

DEVELOPMENT OF MULTI-FACETED IMPLEMENTATION STRATEGY

The implementation strategy was developed systematically and tailored to the determinants of care and the identified barriers for guideline adherence (4, 19). Based on these data, the strategy consisted of 11 elements. For doctors, the elements consisted of a paper and digital version of the revised guideline, a paper and digital short protocol from the guideline, a paper and digital flowchart, an electronic decision program, a pocket card with a point-wise summary of the guideline, and a specified medical chart file for couples with RPL. For patients, we developed a questionnaire about their family history to be filled in prior to their first visit and a patient brochure.

DESIGN

FEASIBILITY

To examine which elements of the strategy are essential in successful implementation, we measured the usage of the elements. We also explored among women and doctors their preferences for the different elements of the strategy.

EFFECTIVENESS

To gain insight into the potential effectiveness of the strategy we performed a study with a before-and-after design. Cohorts consisting of women with RPL were collected both before and after the introduction of the implementation strategy (measurement before strategy: January-December 2006; measurement after strategy: April-September 2009). Nine hospitals participated in the measurement before introduction of the implementation strategy. Results of this measurement have been described

elsewhere in detail (4). The strategy was introduced in four of these nine hospitals in two different regions. Two university hospitals and two non-university teaching hospitals participated. For the purpose of this study, data with regard to before and after measurements of these four hospitals were included. The strategy was introduced in January and February 2009 during a plenary introduction session, where doctors got feedback about their previous guideline adherence. The strategy was explained and all doctors, consultants and registrars, were provided with the 11 elements of the strategy. The measurement of adherence to the quality indicators started three months after the introduction of the strategy.

COST-EFFECTIVENESS

We performed a cost consequence-effectiveness analysis to assess the costs of the development and the actual implementation of the strategy, from a health care perspective. Furthermore, we calculated the direct medical costs for the patients in the situation before and after the introduction of the strategy. Possible effects of the strategy could be a reduction or increase in diagnostic tests, a change in mean consultation time and a change in the mean number of consultations at other specialists.

STUDY POPULATION

For both the before and after measurements regarding effectiveness, all women with a history of two or more miscarriages who had their first visit during the study period were included. They were identified through financial hospitals registries, medical files and clinical genetic registries.

All women included after the introduction of the implementation strategy and their attending doctors were asked for the process evaluation to gain insight into the feasibility of the strategy, that is their preferences for the different elements of the strategy and their usage of the elements.

DATA COLLECTION

FEASIBILITY

To gain insight into the feasibility of the various elements of the implementation strategy from a doctors' perspective, a digital survey was created online (QuestionPro.com). The questionnaire was sent to all doctors who were documented as attending doctor in one or more of the women

included in the study. Use and preferences concerning paper or digital versions of the various elements of the strategy were asked, and possible changes in consultation time. They were asked on a five-point Likert-scale to what extent each element of the strategy was used and to what extent they thought each element was effective for implementation of the guideline. Furthermore, doctors created a top-5 of elements that they consider to be most effective for implementing the guideline, to identify the elements of the strategy with the highest potential for future use. To get insight into the feasibility of the strategy from a patient perspective, the use of the patient questionnaire prior to their visit was documented from the medical files. A paper questionnaire was developed and distributed by mail to the included women. They were asked if they had received a short questionnaire prior to their first visit and if they received an information brochure from their gynaecologist. If so, they were asked how they appreciated both elements and if they had any additional remarks.

Actual use of the short patient questionnaire and prior to the first visit and of the specified medical chart file for RPL were also documented for all patients from their medical files.

EFFECTIVENESS

For the effectiveness evaluation, guideline-based quality indicators were developed just after publication of the Dutch guideline in 2007 (20). The set of quality indicators, covering diagnostics, therapy and counselling for RPL, are an instrument to quantify guideline adherence. Both in the before and after measurement, data needed to establish guideline adherence and patient characteristics, such as obstetric history and family history, was gained from medical records and additional patient questionnaires. Main outcome was the adherence per indicator. The attending doctor was registered for each patient.

COST-EFFECTIVENESS

Throughout the project, all costs associated with the development and the actual implementation of the strategy were assessed using registration forms. All project members (EvdB, MG, JK, FvdV, NL and RH) recorded the travelling hours, travelling costs and number of hours associated with the development of the implementation strategy. The project members also

registered the hours incurred by the specialists to attend focus groups (i.e., part of the development) and introduction meetings (i.e., part of the actual implementation). The costs of medical care before and after introduction of the strategy were assessed by the costs associated with the performed blood tests and consultation time (Payment system DBC).

STATISTICAL ANALYSIS

FEASIBILITY

Descriptive statistics were used to describe usage and preferences of doctors and women with the strategy. To analyse the relationship between indicator adherence and use of the elements, multilevel logistic regression analyses were performed. Corrected for clustering of patients within doctors and the clustering of doctors within hospitals. The first choice of the top five was awarded five points, the second choice four points, the third choice three points, the fourth choice two points and the fifth choice one point. Top 5 score was calculated as the percentage of total point rewarded by all doctors. If all doctors would put the same element on rank 1, this would be a 100% score.

EFFECTIVENESS

Guideline adherence before and after introduction of the strategy was expressed as the percentage of adherence to an indicator, defined as the percentage of women in whom the indicator was followed, divided by the total number of women in whom the indicator should have been followed. The over-all percentage of adherence to the indicators was described as well as the inter hospital range. To test for differences in guideline adherence before and after introduction of the multi-faceted implementation strategy in the four hospitals, both univariable logistic and multilevel logistic regression analysis were performed. The rationale for multilevel regression analysis was the clustering of patients within doctors and the clustering of doctors within hospitals. Multilevel logistic regression analysis per indicator was performed with the percentage of adherence prior to the introduction of the strategy (yes/no) as an independent variable.

COST-EFFECTIVENESS

Time invested by doctors was multiplied by the gross salary (including social premiums and pension contributions) of the persons involved (21). Costs for diagnostics and consultations were reported in 2014 euros with the CPI index obtained from the Statistics Netherlands (CBS). Total costs for diagnostics were calculated for the total group of women before and after the strategy. Average annual saving was extrapolated based on the average number of patients with RPL in the four participating hospitals.

The Statistical Program for the Social Sciences (SPSS for Windows®, SPSS Inc., Chicago, Illinois, USA) and R software (lme4) were used for the analyses. P-values < 0.05 (two-sided) were considered significant.

ETHICS STATEMENT

Subjects did not undergo additional investigations nor treatment. As assessed by the Institutional Review Board (IRB), Academic Medical Centre Amsterdam, the study was not subject to the Dutch 'Medical Research Involving Human Subjects Act' (meaning that no formal IRB approval was needed). Women who lodged an objection to the study were excluded from the study.

RESULTS

We included 599 women in the study, 356 prior to introduction of the implementation strategy and 243 after. From patient questionnaires, which were complementary to the medical files, 300 (50%) were returned fully completed. All 599 women could be included in the analysis with adequate datasets. Baseline characteristics of the patients are presented in Table 1. Baseline characteristics did not differ between women included before and after the strategy, except for the referral pattern. Prior to the strategy more women were already being treated and less women were referred by other specialists compared to population after the introduction of the strategy ($p<0.01$).

Table 1 | Baseline characteristics of the women at time of presentation for recurrent pregnancy loss

	Inclusions prior to implementation n=356	Inclusions after implementation n=243
Maternal age in years[+]	34.5 (5.4)	33.7 (5.1)
Number of preceding miscarriages[√]	2 (1-8)	2 (1-12)
Number of preceding live births[√]	1 (0-4)	1 (0-7)
At least one live birth[∫]	191 (54)	129 (53)
BMI[+]	24.7 (5.0) (n=184)	24.4 (5.0) (n=140)
Referred by[∫*]		
- Self-referral	13 (3.7)	12 (4.9)
- General practitioner	36 (10)	22 (9.1)
- Specialist	37 (10)	50 (21)
- Already being treated[**]	174 (49)	127 (52)
- Other	76 (21)	17 (7.0)
- Unknown	20 (5.6)	15 (6.2)
Nationality[∫]		
- Dutch	180 (51)	126 (52)
- Other	36 (10)	16 (6.6)
- Unknown	140 (39)	101 (42)

+ (mean, SD); √ (median, range); ∫ (n, %); * p< 0.01; ** Patients that were already treated by the attending professional at the time of the diagnosis of recurrent pregnancy loss; BMI: body mass index, SD: standard deviation

MISCARRIAGE

FEASIBILITY

Of the 68 attending professionals, 17 (25%) returned the questionnaire about the strategy. Those professionals together took care of 114 (47%) of the women included after the strategy. Of the 17 professionals, 5 (29%) preferred the elements of the implementation strategy in a paper version, 4 (24%) digital, 5 (29%) a combination of both and 3 (18%) had no specific preference for paper or digital version. The reported use, self-reported effectiveness and top 5 scores are presented in Table 2. The specified medical chart file was used most frequently by the professionals. They chose the "Pocket card" as most useful element for helping to adhere to the guideline. More than 50% of the respondents indicated to intend to use each of the elements in the upcoming year, except for the paper model

protocol and paper flow chart. For the effect of use of the different elements on indicator adherence, the 114 patients treated by the responding professionals could be included. Of the women 100/243 (41%) returned their questionnaire. Fifty-seven (57%) replied they did receive the patient questionnaire prior to their first visit, 30 (30%) that they had not received that questionnaire and 13 women (13%) did not remember whether they received it. Of the 57 women that received the questionnaire, 45 (79%) found the short questionnaire useful, one (2%) found it not useful and 11 (19%) had no opinion. Thirty-eight women (38%) received the patient brochure, 40 (40%) did not receive it and 22 (22%) did not remember. Of the 38 women who received the brochure, 33 (87%) found it useful, four (10%) found it not useful and one (3%) had no opinion on whether it was useful or not.

Table 2 | Use and Top 5 ranking elements from the implementation strategy

	Reported use*#	Reported effectiveness+#	Top 5 score∫
Pocket card	1.5 (1-5)	2.5 (1-4)	19
Guideline digital	2.0 (1-5)	3.0 (1-4)	16
Specified medical chart file	2.5 (1-5)	2.5 (1-4)	14
Flowchart digital	1.0 (1-5)	2.5 (1-4)	11
Patient questionnaire	1.0 (1-5)	3.0 (1-4)	10
Guideline paper	2.0 (1-5)	3.0 (1-4)	8
Electronic decision program	1.0 (1-4)	3.0 (1-4)	7
Patient brochure	1.0 (1-5)	2.0 (1-4)	6
Flowchart paper	1.0 (1-5)	1.5 (1-4)	5
Modelprotocol digital	1.0 (1-5)	1.5 (1-4)	4
Modelprotocol paper	2.0 (1-5)	1.5 (1-4)	0

* Scored on a 5-point Likert scale: 1= Never used, 5= Used in almost all patients; + Scored on a 5-point Likert scale: 1= Not effective, 5= Very effective; # median with range between parentheses;

∫ Top 5 ranking of most effective tools. Rank 1 = 5 points, rank 2 = 4 points, rank 3 = 3 points, rank 4 = 2 points, rank 5 = 1 point. Presented is the percentage of total point rewarded by all professionals. If all professionals put the same tool on rank 1, a 100% score would be rewarded. If none of the professionals mentioned the tool in their top 5, the score would be 0%.

The relationship between the (reported) use of the elements and the adherence per indicator is reported in Table 3. Results are shown for indicators directly related to diagnostic tests, which are related to cost-reduction. The specified medical chart file, patient questionnaire, pocket card and electronic decision program were, in varying combination, related to higher adherence to diagnostic indicators. For the other indicators, no relationship with use of the elements was found, or could not be measured due to low patient numbers.

Table 3 | Multilevel logistic regression analysis for use of elements per indicator related to increase in adherence

	Adjusted OR
*Total number of objectified miscarriages defined**	
Specified medical chart file	4.2 (2.0 – 9.1)
Patient questionnaire	3.5 (1.6 – 7.9)
Selective karyotyping	
Specified medical chart file	2.6 (1.3 – 7.1)
Electronic decision program	1.8 (1.2 – 4.1)
Patient questionnaire	2.3 (1.1 – 5.9)
Antiphospholipid antibodies determined	
Pocket card	4.5 (1.2 – 7.3)
Homocysteine determined	
Pocket card	4.9 (1.8 – 9.1)
Specified medical chart file	5.3 (2.1 – 9.0)
Calculation of pregnancy success in next pregnancy	
Electronic decision program	2.5 (1.1 – 4.8)

* Information available on specified medical chart file and patient questionnaire in all patients. Use of other elements known in n=114 patients; OR: Odds Ratio

EFFECTIVENESS

The adherence per indicator before and after the introduction of the strategy is presented in Table 4. For diagnostic indicators the highest increase in adherence was measured for selective thrombophilia screening (+ 37%, OR 5.2, 95% CI 3.6-7.6). Maternal age at the time of the second miscarriage was reported 32% more often after than before the strategy (OR 8.2, 95% CI 5.3-13). Adherence to selective karyotyping increased significantly from 50% before up to 76% after the strategy (OR 3.3, 95% CI 2.2- 4.6).

Table 4 | Actual care measured per quality indicator for recurrent pregnancy loss

Quality indicator	Before introduction of the strategy		After introfuction of the strategy		Difference in adherence (%)	OR (CI 95%)
	Indicator followed	Adherence (%)	Indicator followed	Adherence (%)		
Medical history[+]						
Lifestyle	282/356	80	195/243	80	-	1.1 (0.7–1.7)
History of thrombophilia	243/356	68	197/243	81	13	1.7 (1.1–2.4)
Number of objectified miscarriages	217/356	61	159/243	65	4	1.3 (0.9–1.8)
Family history of RPL	171/356	48	144/243	59	11	1.6 (1.2–2.3)
Maternal age 2nd miscarriage	32/356	9	108/243	41	32	8.2 (5.3–13.0)
Family history on thrombophilia	9/356	2.5	133/243	55	53	Not available
Diagnostics						
Antiphospholipid antibodies	197/356	55	153/243	63	8	1.4 (1.0–2.0)
Karyotyping (selectively)	177/356	50	185/243	76	26	3.3 (2.2–4.6)
Homocysteine	176/356	49	158/243	65	16	1.9 (1.4–2.7)
Thrombphilia factors (select)	158/356	44	195/243	81	37	5.2 (3.6–7.6)
Length, weight, BMI	47/356	13	75/243	31	18	3.0 (2.0–4.6)

Quality indicator	Before introduction of the strategy		After introduction of the strategy		Difference in adherence (%)	OR (CI 95%)*
	Indicator followed	Adherence (%)	Indicator followed	Adherence (%)		
Therapy						
Supplement vitamins in low levels	2/2	100	0/0	-	-	Not available
Withhold immunological therapy	356/356	100	243/243	100	0	Not available
Prescribe aspirin/LMWH in APS	2/2	100	5/5	100	0	Not available
Refer to geneticist	7/7	100	3/3	100	0	Not available
Withhold aspirin in uRPL	327/331	99	221/224	99	0	Not available
Experimental treatment only in RCT	325/331	98	223/224	99	0	Not available
Counselling/Advice						
Carrier couples on high success rate	4/7	57	3/3	67	10	Not available
Quit smoking	7/61	12	31/50	62	50	Not available
Weight loss	14/66	21	30/51	59	38	5.3 (2.4–12.0)
TLC	Not measurable					
Discuss success rate uRPL	62/331	19	60/224	27	8	1.6 (1.1–2.4)
Folic acid use	Not measurable					

* Multilevel analysis Odds Ratio (OR); + Reporting of indicators of medical history; RPL: recurrent pregnancy loss; BMI: body mass index; LMWH: low molecular weight heparin; APS: antiphospholipid syndrome; uRPL: unexplained recurrent pregnancy loss; RCT: randomized controlled trial;

Significant increase in adherence was also seen for determination of Body Mass Index (BMI) (+18%, OR 3.0, 95% CI 2.0-4.6), homocysteine (+16%, OR 1.9, 95% CI 1.4-2.7) history of thrombophilia (+13%, OR 1.7, 95% CI 1.1-2.4), family history of RPL (+11%, OR 1.6, 95% CI 1.2-2.3) and antiphospholipid antibodies (+8%, OR 1.4, 95% CI 1.03-2.0). For the indicator to report the number of objectified pregnancy losses a trend towards increase of adherence was seen but these indicators did not reach significance. Report on lifestyle remained 80%. None of the indicators showed a decrease in adherence. Report on family history of thrombophilia showed the highest increase in adherence (+53%), but could not be included in multilevel analyses due to a small number of patients (n=9) prior to the introduction of the strategy. For the indicators on therapy, none of the indicators showed a significant increase (before the strategy adherence was almost 100%) or decrease in adherence. Variation in adherence between the different hospitals both before and after the strategy was very small. For the indicators on counselling, for two out of four measurable indicators an increase in adherence was observed after the strategy: Advise weight loss (+38%, OR 5.3, 95% CI 2.4-12), and discuss individual chances on reproductive outcome in unexplained RPL (+8%, OR 1.6, 95% CI 1.1-2.4). The indicator advises patient and partner to quit smoking increased with 62%, and the indicator to inform carrier couples about good reproductive chances showed an increase in adherence of 10%. Multilevel analyses were not possible for these indicators due to the small number of patients.

COST-EFFECTIVENESS

The costs were 69,028 euros for the development of the implementation strategy and 19,325 euros for the actual implementation. So, the over-all costs were 88,353 euros. Costs for the development consisted of personnel costs of the project group (60,254 euros), travelling costs (personnel and travelling budget: 1,562 euros) and costs for focus groups (7,212 euros). Cost for the actual implementation included for six introduction meetings that resulted in 127 personnel hours with a total cost of 7,457 euros (travelling costs included), other travelling costs (personnel and travelling budget: 806 euros), and costs for dissemination of the elements of the strategy (11,063 euros).

In addition, the mean time for a specialist to study the digital and/or the paper version of the implementation package was 14 minutes. One of the changes in costs of medical care before and after introduction of the strategy included the consultation time. Professionals indicated to use on average an estimated 18 minutes per consultation for RPL before the implementation package was introduced (range 10-45 minutes). After the introduction of the implementation strategy, professionals indicated to use on average an estimated 16 minutes per consultation (range 8-30 minutes). The cost of a consultation was fixed at 112 euros. When professionals were asked about changes in the number of consults required for RPL patients since the introduction of the implementation strategy, nine professionals (53%) reported having needed less consultations, while eight (47%) did not recognize a change in the number of consults. Changes in costs of medical care before and after introduction of the strategy were as follows (Table 5). During the 6 months study period after the strategy, a reduction of 91,892 euros was achieved. In the four participating centres, 535 couples with RPL were seen annually, which would have resulted in a saving of 206,916 euros.

DISCUSSION

Guideline adherence in couples with RPL was improved after introducing a tailored multifaceted implementation strategy. Prior to the strategy, 9 out of 21 measurable indicators showed an adherence below 50%. After the implementation strategy, adherence was below 50% for just three out of 21 indicators. Adherence increased significantly in ten indicators, mainly on diagnosis. For two indicators we observed a trend towards increase of adherence, but the confidence intervals for these indicators did not reach significance. For none of the indicators a decrease in adherence was observed.

The "Specified medical chart file" was used most by professionals. Professionals chose the "Pocket card" as most useful element of the strategy to improve guideline adherence. The measured as well as the self-reported use of the various elements were related to a better adherence to the guideline compared to the use of the other elements – the ones used less. Thirty-five percent of the doctors reported that fewer consultations were needed after the introduction of the implementation strategy and over 79%

of the women appreciated the patient related elements of the strategy; The specified medical chart file, patient questionnaire, pocket card and electronic decision support instrument significantly helped to improve the quality of care delivered.

Table 5 | Cost effectiveness of the developed implementation strategy

Diagnostic test	Diagnostic test performed before/after implementation*				Cost change across 4 centres+	
	Before	After	Difference	Cost of test*	6 months	12 months
Karyotyping	74%	52%	-22%	€1.664,-	-€86.987,-	-€193.498,-
APS	55%	63%	+8%	€36,-	+€697,-	+€1.541,-
Homocysteine	49%	65%	+16%	€39,-	+€1.510,-	+€3.338,-
Thrombophilia#	56%	20%	-36%	€95,-	-€8.276,-	-€18.297,-
				Total	-€93.056,-	-€206.916,-

* percentage of couples that received the diagnostic test before and after implementation strategy; + change in costs of diagnostic testing compared to before implementation strategy, calculated by multiplying number of couples that received the tests by the cost per diagnostic test; # antithrombin III, protein C, protein S, factor V Leiden, factor II, factor VIII ; APS: antiphospholipid syndrome (anticardiolipin antibodies IgG and IgM, lupus anticoagulant);

Regarding the costs, a reduction of 206,916 euros per annum for the four participating hospitals together was achieved. If the implementation strategy was applied throughout The Netherlands even higher annual savings would be expected, due to the lack of further developmental costs and expected lower costs of the introduction of the strategy. It is difficult to indicate the exact number of couples with RPL per year in The Netherlands, since it is a condition that is not registered on a national level. In our measurement prior to the strategy, 72% of all new couples were karyotyped. Annually, 1470 couples with RPL are karyotyped in The Netherlands (registries by genetical testing centres in the Netherlands). When extrapolated, an estimated number of 1,900 couples are seen per year with RPL. This indicates a cost reduction of at least 791,367 euros per year in The Netherlands.

The most important strength of our study is the structured development of the strategy. We incorporated the results of the measurement of actual care, the determinants for non-adherence and the results from barriers and facilitators (18). By testing in two different regions in the country, local cultural differences were covered, increasing the potential for wider use of the strategy. Also, this is one of the first studies that actually related the effect of the strategy on guideline adherence to the use of the various elements of that strategy by the professionals involved. This step is necessary to know which elements are actually the effective ones, and useful for future implementation, to make it more effective and less expensive (22-24). The cost-effectiveness analysis includes costs of the development as well as the use and effect of the strategy, which gives a realistic perspective of the actual costs in daily clinical practice.

Some limitations should be discussed while interpreting the results. Due to the method of before and after measurement used in our study, the results only present a potential effect of the strategy on guideline adherence. The exact strategy-related effectiveness should be measured within the setting of a double blind randomized clinical trial (RCT)(25). Such a RCT is difficult, since a complete non-intervention group is hard to accomplish. In other words, the quality of care is the outcome measurement and attention of professionals for the guideline alone is already a first to attempt towards implementation. The response rate for the feasibility study was quite low

among doctors and patients, which might explain and the wide 95% CI, described in Table 1.

Even though the results are promising, caution is needed in interpreting the results in current practice. As the implementation strategies were implemented and carried out between 2006 and 2009, they reflect on a different era, in which protocols with electronical availability were not yet available automatically. Nevertheless, in our view this does not invalidate the results of our study, since the technological developments will lead to easier access to the various elements of the strategy. For example, a digital patient file could be designed with a customized module for couples with RPL. Thereby incorporating the specified medical chart file and electronic decision program in standard patient care.

Revised international guidelines in RPL are about to be published. Within the field of reproductive medicine, implementation strategies to improve guideline adherence were tested with varying success (16, 17). A gold standard for implementation strategies does not exist but our results underscore that a strategy should be tailored to the results of the actual care measurement, the determinants of care and the identified barriers for guideline adherence (14, 26).

Recently, the ESHRE stated that implementation tools are important, although there is little evidence for their efficacy, and that current implementation strategies are lacking (27). This study provides clear evidence for the efficacy of implementation strategies, as portrayed in the high cost -reduction.

The ESHRE guideline on RPL is currently under revision and we encourage that its publication – as well as future revisions- should be accompanied with electronical implementation tools which were effective in our strategy, to optimize a prompt implementation of this revised guideline.

CONCLUSION

Robust implementation strategies are necessary to achieve proper adherence in RPL care. A multi-faceted implementation strategy was tested, showing that implementation strategies are feasible, effective in increasing adherence and could lead to cost-reductions. This implementation strategy can widely be introduced in clinical practice for RPL, and may serve as an example for future implementation strategies in other areas within obstetrics and gynaecology.

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PART II

PROGNOSIS IN RECURRENT PREGNANCY LOSS

6

CHAPTER 6

DEFINING RECURRENT PREGNANCY LOSS; ASSOCIATED FACTORS AND PROGNOSIS IN COUPLES WITH TWO VS THREE OR MORE PREGNANCY LOSSES

A. Youssef, E.E.L.O. Lashley, S.W.M. Dieben, H.J. Verburg and M.L.P. van der Hoorn

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ABSTRACT

RESEARCH QUESTION

The definition of recurrent pregnancy loss (RPL) differs internationally. The European Society of Human Reproduction and Embryology (ESHRE) defines RPL as two or more pregnancy losses. Different definitions lead, however, to different approaches to care for couples with RPL. This study aimed to determine whether the distribution of RPL-associated factors was different in couples with two versus three or more pregnancy losses. If a similar distribution were found, couples with two pregnancy losses should be eligible for the same care pathway as couples with three pregnancy losses.

DESIGN

This single-centre, retrospective cohort study investigated 383 couples included from 2012 to 2016 at the Leiden University Medical Centre RPL clinic. Details on age, body mass index, smoking status, number of pregnancy losses, mean time to pregnancy loss and performed investigations were collected. The prevalence of uterine anomalies, antiphospholipid syndrome, hereditary thrombophilia, hyperhomocysteinemia, chromosomal abnormalities and positive thyroid peroxidase antibodies were compared in couples with two versus three or more pregnancy losses.

RESULTS

No associated factor was found in 71.5% of couples with RPL. This did not differ statistically between couples with two versus three or more pregnancy losses (73.6% versus 70.6%; p=0.569). The distribution of investigated causes did not differ between the two groups.

CONCLUSIONS

As the distribution of associated factors in couples with two versus three or more pregnancy losses is equal, couples with two pregnancy losses should be eligible for the same care pathway as couples with three. This study supports ESHRE's suggestion of including two pregnancy losses in the definition of RPL.

INTRODUCTION

Miscarriage is defined as the spontaneous loss of conception before the 24th week of gestation, and occurs in approximately 15% of pregnancies (1). Recurrent pregnancy loss (RPL) was recently defined by the "European Society of Human Reproduction and Embryology" (ESHRE) as two or more pregnancy losses (1). This new definition includes biochemical pregnancies and pregnancies of unknown location. The prevalence of a spontaneous loss could be higher using the ESHRE definition (2). However, different definitions are used in different guidelines and different countries (1, 3).

Factors generally accepted to be associated with RPL include uterine malformations, maternal antiphospholipid syndrome (APS), maternal thrombophilia, endocrine disease (such as diabetes and presence of thyroid antibodies), autoimmune diseases and parental structural chromosomal abnormality (4). Factors contributing to RPL are increased female age, female weight (obesity and underweight) and lifestyle factors such as smoking, caffeine and alcohol intake (5). Unfortunately, in approximately 50% of couples no associated factor can be identified (6).

In the discussion of defining RPL, Boogaard et al stated that the number of preceding miscarriages is not associated with the risk of APS and that APS testing should also be considered for women with two or more pregnancy losses (7). The same authors showed that the probability of carrier status of a structural chromosomal abnormality is not only influenced by the number of preceding miscarriages. Low maternal age at second miscarriage, a history of two or more miscarriages in a brother or sister of either partner, and a history of two or more miscarriages in the parents of either partner do increase the probability of carrier status (8).

However, whether the risk related to the above-mentioned associated factors is different for couples with two versus three or more pregnancy losses remains to be elucidated. This is important to know because if a similar distribution of these factors were found, couples with two pregnancy losses should be eligible for the same care pathway as couples with three or more pregnancy losses. It could also be of use in clinical research as varying definitions are currently used. In addition, it is not known whether the risk of a subsequent miscarriage after two (non-

)consecutive losses is similar to the risk after three miscarriages (9, 10). From a patient's perspective, this question might be even more important.

The objective of this study was therefore to determine whether the distribution of RPL associated factors is different in couples with two vs three or more pregnancy losses. In addition, mean gestational age at time of miscarriage and chance of future ongoing pregnancy were assessed.

MATERIALS AND METHODS

This single-centre retrospective cohort study included couples with a history of RPL who were evaluated at the RPL clinic of the Leiden University Medical Centre (LUMC) between November 2012 and October 2016. The clinic investigates associated factors in couples with RPL and provides support during subsequent pregnancies, with weekly ultrasounds from the sixth week of gestational age until the 12th week.

RPL was defined as at least two or more pregnancy losses before 24 weeks of gestational age, including non-visualized pregnancies and non-consecutive pregnancy losses. Ectopic and molar pregnancies were not included in the study definition. An ongoing pregnancy was defined as a pregnancy continuing after the 10th gestational week.

A database was created in SPSS (IBM Inc., New York, USA version 23) to include data from the electronic patient records on intake of each new patient who visited the clinic in the defined period. Clinical data prior to 2013 originated from paper files. At the first intake appointment, a thorough medical and obstetric questionnaire was completed and women were evaluated for uterine anomalies, APS (anticardiolipin antibodies, lupus anticoagulant and anti-β2-glycoprotein-I), hereditary thrombophilia (protein C, protein S or antithrombin III deficiency, activated protein C [APC] resistance [due to the factor V Leiden mutation] or factor II mutation), hyperhomocysteinemia and parental karyotyping. Parental karyotyping was evaluated according to a priori probability (8), APS and maternal thrombophilia testing was performed at least 12 weeks apart from a patient's last miscarriage. Details of the procedure for evaluating the causes of RPL are described below. Anti-thyroid peroxidase (anti-TPO) concentrations were measured in patients interested in participating in a

clinical trial (T4-LIFE) studying the effectiveness of levothyroxine supplementation in women with RPL and thyroid autoimmunity (11).

The first day of the last menstrual period was used to calculate the gestational age of the miscarriage as exact methods, such as ultrasonography, were not available for all women. The outcome and parameters of the subsequent pregnancy were documented, and information collected from medical records outside the hospital was added to the patient file and database. The results of investigation of RPL for all couples were discussed in an RPL team. Consensus was achieved in diagnosing possible causes of the RPL. RPL was declared unexplained when none of the investigated causes was found in couples.

EVALUATION OF CAUSES OF RPL

Congenital uterine anomalies were detected by 3D ultrasound in the luteal phase. The 3D ultrasound was performed by a sonographer specialised in 3D ultrasounds. Diagnostic methods, for example hysteroscopy, used outside the centre were added to the database. The ESHRE/ESGE classification of female tract congenital anomalies was used to classify uterine anomalies (18). Uterine anomalies were already being diagnosed in coherence with the guideline before its publication.

APS was diagnosed if at least one clinical and one laboratory criterion was present (19). The clinical criteria were either vascular thrombosis or pregnancy morbidity (including unexplained RPL); laboratory criteria were the presence of lupus anticoagulant, elevated titres of anticardiolipin IgG and/or IgM antibody (≥40 U/ml), or anti-β2 glycoprotein-I IgG and/or IgM antibody (≥17 U/ml) at two different time points at least 12 weeks apart. Women with a positive diagnosis were given low molecular weight heparin (2850 IU daily), starting from a positive pregnancy test up to 24 h before labour. Aspirin (80 mg daily) was added once a foetal heartbeat was detected and was continued up to 36 weeks' gestational age.

Abnormal results were recorded when antigen concentrations and activity levels of protein C were less than 66% and 64%, respectively. A free protein S antigen concentration less than 0.53 IU/ml and an antithrombin III antigen concentration less than 84% were considered abnormal. Factor II mutations (heterozygous/homozygous) were investigated, as was the factor

V Leiden mutation when the APC resistance ratio was lower than 0.91. Hyperhomocysteinemia was diagnosed when random homocysteine concentrations surpassed 15 mmol/l. If homocysteine concentrations were elevated, vitamin B6 (reference range 54–136 nmol/l) and B12 concentrations (reference range 150–700 pmol/l) were measured; supplements were given if the levels were too low. After 6 weeks, the homocysteine concentration was re-evaluated. If it was normal, couples could stop using contraceptives.

According to Franssen's risk table on chromosomal abnormalities, couples could be genetically tested for the presence of structural chromosomal abnormalities (8). A clinical geneticist evaluated both parents' karyotype and concluded whether significant abnormalities were present.

ETHICAL APPROVAL

The study protocol (reference number P11.196) was approved by the LUMC ethics committee (October 2015).

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS Statistics version 23 (IBM, USA). To analyse differences between the groups, the independent samples t-test was used for continuous data. For categorical variables, Pearson's chi-squared test was used. If there was an expected count of less than 5 in 20% or more of all cells in SPSS, Fisher's exact test was used.

A linear by linear association chi-squared test was used to compare the chances of ongoing pregnancy in couples with different gestational ages. Binary logistic regression analysis was conducted to assess the influence of age and body mass index (BMI) on the chance of having a future ongoing pregnancy. BMI was used as a categorical variable, with BMI categories of less than 20 kg/m^2, over 25 to 30 kg/m^2 and over 30 kg/m^2 compared with the healthy range of 20–25 kg/m^2. Groups were assumed to differ significantly when the probability level was less than 0.05.

RESULTS

Over the study period in 2012 until 2016, 383 couples with at least two pregnancy losses visited the clinic and were assessed. The women's mean age was 33.7 years and mean BMI 25.1 kg/m^2. The mean gestational age of

the pregnancy loss at presentation was 7 weeks and 4 days. The percentage of women who smoked was 17.3%. Table 1 displays the baseline characteristics of the RPL population. Due to missing data, the number of couples used in calculating the baseline characteristics is given for BMI, mean gestational age, smoking rate and ongoing pregnancy rate.

Table 1 | Baseline characteristics

	Total	Two pregnancy losses	Three or more pregnancy losses	p-value+
Age (years), mean ± SD (n)	33.7 ± 4.7 (383)	33.6 ± 4.7 (107)	33.7 ±4.7 (276)	0.800
BMI (kg/m^2), mean ± SD (n)	25.1 ± 5.2 (332)	25.4 ± 5.2 (91)	25.0 ± 5.1 (241)	0.583
Prior live birth, % (n)	42.6 (163/383)	51.4 (55/107)	39.1 (108/276)	0.029
Gestational age* (weeks), mean ± SD (n)	7.6 ± 1.6 (324)	8.0 ± 1.7 (84)	7.4 ± 1.5 (240)	0.004
Smokers, % (n)	17.3 ± 3.8 (307/371)	16.8 ± 3.8 (17/101)	17.4 ± 3.8 (47/270)	0.896
Ongoing pregnancy rate, % (n)	83.5 (212/254)	91.5 (65/71)	80.3 (147/183)	0.031

SD: standard deviation
* Mean gestational age at time of miscarriage
+ Independent samples t-test

At the patients' initial visit, for the mean age of 33.7 years (n=383), the age range was 22–45 years. An independent t-test showed no statistically significant difference in age among women with two versus three or more pregnancy losses (mean age 33.6 ± 4.7 years (n=107) for two losses and 33.7 ± 4.7 years (n=276) for three or more losses; p=0.800). Mean BMI (kg/m^2) did not differ between women with two versus three or more losses (25.4 ± 5.2 versus 25.0 ± 5.1 kg/m^2; independent t-test, p=0.583), and neither did the smoking percentage (16.8 ± 3.8 versus 17.4 ± 3.8; chi-squared test, p=0.896).

The majority of women (98.2%, n=324) had a miscarriage before the 11th week of gestation. The mean gestational age at pregnancy loss was significantly higher in women with two versus three or more pregnancy losses (8.0 ± 1.7 (n=84) versus 7.4 ± 1.5 (n=240); independent t-test, p=0.004).

ASSOCIATED FACTORS OF TWO VS THREE OR MORE PREGNANCY LOSSES

At the initial visit, 27.9% (n=107) of the couples had two pregnancy losses and 72.1% (n = 276) had three or more pregnancy losses. Almost half of all couples (42.6%, n=163) had at least one live birth prior to or in between the pregnancy losses.

Table 2 summarizes the distribution of associated factors among these couples.

Table 2 | Distribution of associated factors

	Two pregnancy losses	Three or more pregnancy losses	Total	p-value[+]
Hyperhomocysteinemia, % (n)	2.0 (2/101)	1.1 (3/265)	1.4 (5/366)	0.619[*]
Antiphospholipid syndrome, % (n)	6.5 (7/107)	7.6 (21/276)	7.3 (28/383)	0.719[+]
Hereditary thrombophilia, % (n)	12.7 (13/102)	11.8 (31/263)	12.1 (44/365)	0.801[+]
Chromosomal abnormality, % (n)	2.9 (2/68)	2.9 (5/172)	2.9 (7/240)	1.000[*]
Anti-thyroid peroxidase, % (n)	9.0 (7/78)	10.7 (18/168)	10.2 (25/246)	0.674[+]
Uterine anomaly, % (n)	3.4 (3/88)	7.1 (17/239)	6.1 (20/327)	0.215[+]
Unexplained, % (n)	73.6 (78/106)	70.6 (190/269)	71.5 (268/375)	0.569[+]

* Fisher's exact test
\+ Chi-squared test

In 71.5% of 375 couples who were evaluated, RPL was unexplained. Due to missing data, we were not able to register a diagnosis for eight couples in the database. The prevalence of investigated factors in couples with two vs. three or more pregnancy losses was: uterine anomalies, 3.4% vs. 7.1% (p=0.215); APS, 6.5% vs. 7.6% (p=0.719); hereditary thrombophilia, 12.7% vs. 11.8% (p=0.801); hyperhomocysteinemia, 2.0% vs. 1.1% (p=0.619); chromosomal abnormalities, 2.9% vs. 2.9% (p=1.000); and positive TPO antibodies, 9.0% vs. 10.7% (p=0.674). The distribution of associated factors did not differ between the two groups. There was no statistically significant difference in the number of unexplained RPL (73.6% versus 70.6%; p=0.569) in couples with two versus three or more pregnancy losses.

PROGNOSTIC FACTORS

Of the 383 couples included in this study, 66.3% (n=254) continued to visit the LUMC with a new pregnancy after their RPL workup and treatment advice plan. The mean follow-up time was 37.7 ± 7.1 months. Of these couples, 83% (n=212) had an ongoing pregnancy during follow-up. Couples with two pregnancy losses had significantly more ongoing pregnancies compared with couples with three or more losses (91.5% vs. 80.3%; p=0.031; Table 1). This decrease in number of ongoing pregnancies continued when two, three or four or more losses were compared, as shown in Figure 1 (p=0.035 for overall decrease from two until four or more losses).

As the mean gestational age of miscarriage increased, an associated factor was more often identified (p=0.004) and the chance of having an ongoing pregnancy increased (p=0.005) (Figure 2). In contrast to couples with two losses, the association between the mean gestational age of the miscarriage and the chance of having an ongoing pregnancy was significantly different in couples with three or more pregnancy losses (chi-squared test, p=0.458 vs. p=0.035).

A greater number of women with a BMI of 25 kg/m^2 or less compared with a BMI of over 25 kg/m^2 had an unexplained cause of RPL (n=330, 72.3%, 138/191, vs. 60.4%, 84/139; p=0.024). This difference remained statistically significant in women with two pregnancy losses (n=90, 76.6%, 36/47, versus 51.2%, 22/43; p=0.012) but not in women with three losses (n=235, 70.4%, 100/142, vs. 63.4%, 59/93; p=0.263). The distribution of associated factors was not statistically significantly different in the two comparisons.

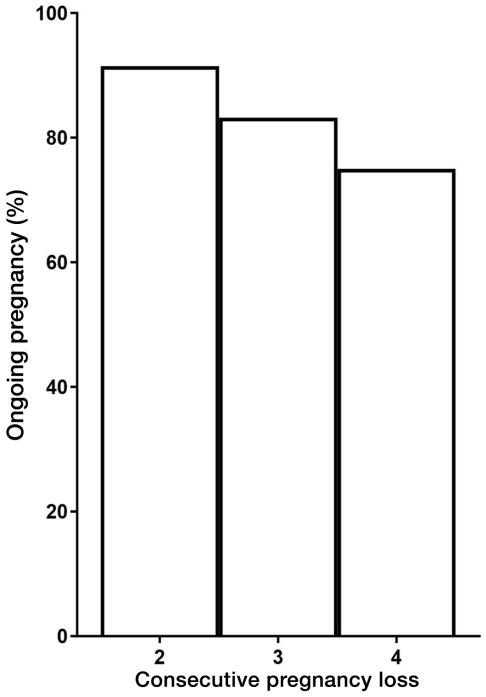

Figure 1 The chance of a future ongoing pregnancy in women with two, three or four or more pregnancy losses. Total, n = 254; two losses, n = 71; three losses, n = 119; four or more losses, n = 64. Chi-squared test, P = 0.035 for overall decrease from two until four or more losses.

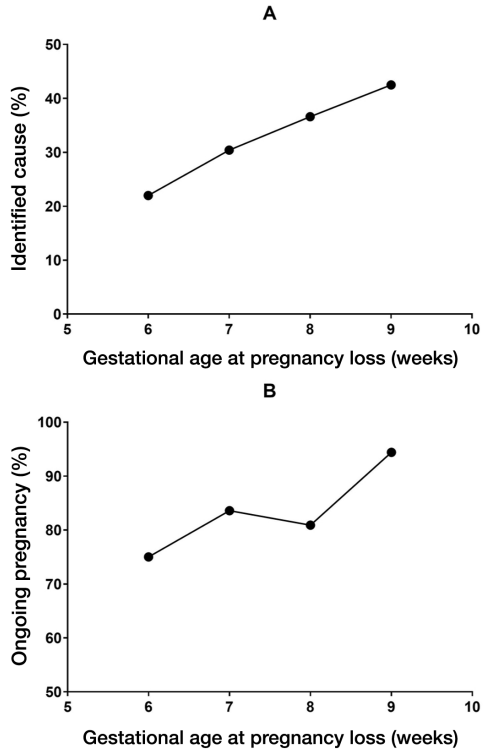

Figure 2 Prognostic factors. (A) Graph showing how often an associated factor was found in women with different mean gestational ages at the time of miscarriage. Chi-squared test, p=0.004 (for overall graph). (B) Graph showing the chance of a future ongoing pregnancy in women with different mean gestational ages at the time of miscarriage. Chi-squared test, p=0.005 (for overall graph).

Table 3 | Analysis of the influence of age (years) and BMI (kg/m^2) on the chance of having a future ongoing pregnancy in women followed up after their first visit

				95% CI	
	B ± SE	**p-value**	**OR**	**Lower**	**Upper**
Age	−0.023 ± 0.041	0.574	0.977	0.901	1.060
BMI 20–25		0.265			
BMI <20	0.655 ± 0.589	0.266	1.926	0.607	6.107
BMI >25 to 30	0.787 ± 0.470	0.094	2.197	0.875	5.521
BMI >30	0.698 ± 0.587	0.235	2.009	0.636	6.353
Constant	2.020 ± 1.415	0.153	7.539		

Body mass index (BMI) was used as categorical variable, in which the categories BMI <20 kg/m^2, over 25 to 30 kg/m^2 and over 30 kg/m^2 were compared with the healthy range of 20–25 kg/m^2.
B: unstandardized regression weight; OR, odds ratio for the correspondent variable with regard to the chance of having a future ongoing pregnancy.

The binary logistic regression analysis did not indicate an association between age, BMI and the chance of an ongoing pregnancy (Table 3). This did not change when the analysis was performed separately in women with two or with three or more pregnancy losses.

DISCUSSION

In this study, associated and prognostic factors were compared between couples with two and those with three or more pregnancy losses in 383 couples from the RPL cohort over a period during 2012–2016. Factors associated with RPL occurred with equal frequency in women with two versus three or more pregnancy losses.

A knowledge of similarities between couples with two versus three or more pregnancy losses is clinically relevant. A uniform definition is of importance to be able to standardize protocols as well as for a unified method in scientific research related to women with RPL. These women carry a burden of not being able to successfully reproduce, and early intervention with "tender, loving care" could reassure these couples. Reassurance could also be derived from information on the chances of future ongoing pregnancy.

In most cases (71.5%), no underlying associated factor of RPL was found. The most common factor was thrombophilia, occurring in 12.1% of RPL couples, followed by anti-TPO antibodies (10.2%), APS (7.3%), uterine malformations (6.1%), chromosomal abnormalities (2.9%) and hyperhomocysteinemia (1.4%). The prevalence of these associated factors was compared with other studies, and no striking differences were found (7, 12, 13). The most important difference is the inclusion of anti-TPO in the RPL workup in the current study. Anti-TPO is associated with RPL and the odds of pregnancy losses are increased in women with antibodies, even if they have normal thyroid function. Couples willing to participate in the T4-LIFE study, which investigates levothyroxine supplementation in women with RPL and thyroid autoimmunity in relation to live birth rate and pregnancy outcome, were assessed for the presence of autoantibodies directed against the thyroid gland.

In the current study, women with two versus three or more pregnancy losses were comparable in their characteristics in terms of age, BMI and smoking habit. The distribution of associated factors was equal in couples

with two versus three or more pregnancy losses, and a lack of cause was found as often in the two groups. The mean gestational age of the miscarriage was, however, significantly lower in the group with at least three pregnancy losses. It is well known that foetal genetic abnormalities occur frequently and can be the cause of a pregnancy loss (14, 15). In women with three or more pregnancy losses, these pregnancy losses could potentially be explained by foetal genetical abnormalities, leading to miscarriage at earlier gestational age. Although the result was highly statistically significant, the difference was one of only a few days, and one could argue the clinical relevance of this observation.

The chances of having a future ongoing pregnancy are relatively high even though this chance decreases with an increasing number of pregnancy losses. During follow-up, 83.5% of women were reported to have an ongoing pregnancy, which is in line with another cohort of women with unexplained RPL (16). Also in accordance with this, a subsequent pregnancy loss was seen to negatively influence the chance of a future ongoing pregnancy. In the current study, the chance of a future ongoing pregnancy was calculated in relation to the whole RPL population, whereas Brigham and colleagues calculated this chance in a population of women with unexplained RPL. The chance of a future ongoing pregnancy is, however, still relatively high (Figure 1), which could be a comforting thought for women suffering from RPL.

This study collected data from women who visited the RPL clinic between 2012 and 2016, even though the clinic opened in 2007. Data originating before 2013 were derived from paper files, so were less accessible than if electronic patient files had been used. Moreover, the data from the paper files were frequently incomplete, but adding this information through questionnaires could have led to recall bias. However, the data derived from paper files formed the minority of the collected data. Many women had been referred to the clinic having undergone several investigations elsewhere, meaning that information from those investigations was added to the database.

This is, for example, the case in the investigation of uterine anomalies. In other hospitals, general 2D ultrasound, hysteroscopy, hysterosalpingography, laparoscopy, magnetic resonance imaging or saline

infusion sonohysterography were used to diagnose anomalies, whereas the LUMC protocol uses a 3D ultrasound technique. This could have led to an underestimation of the prevalence of uterine anomalies in the current cohort.

New insights into hyperhomocysteinemia and RPL are presented in the latest ESHRE guideline. Before this guideline, hyperhomocysteinemia was part of the regular RPL investigations. The results of statistical testing did not, however, change when hyperhomocysteinemia was excluded from the analysis.

Another important limitation of this study is the small number of couples included. A larger cohort is needed to estimate the outcomes more precisely, especially for prognostic factors.

Jaslow and colleagues showed that the distribution of associated factors in women with RPL did not differ between two, three or four or more losses (12). In addition, Bashiri and co-workers showed that there were no differences between women with two versus three or more losses (13). Van Dijk and colleagues recently published a systematic review and meta-analysis evaluating the occurrence of abnormal test results in patients with two versus three or more pregnancy losses (17). They concluded that there was no difference in the prevalence in uterine abnormalities and APS, but that they could not exclude a difference in chromosomal abnormalities, inherited thrombophilia and thyroid disorders.

The current findings are in accordance with this review. Moreover, the current study adds to the body of evidence that there is no difference in inherited thrombophilia in women with two versus three or more pregnancy losses. In this cohort, the number of chromosomal abnormalities was equal, but numbers were small. With regard to thyroid diseases, anti-TPO results are equal in both groups in this study. Again, the number of patients with TPO antibodies is small, as the presence of these antibodies was, as mentioned above, only assessed in couples who were willing to participate in the T4-LIFE study.

CONCLUSION

This study assessed differences in factors associated with RPL in women with two versus three or more pregnancy losses in terms of the discussion of the definition of RPL. An equal distribution of associated factors was found in couples with two versus three or more pregnancy losses. The most recent ESHRE guideline advises defining RPL as starting from two pregnancy losses, and this study supports that definition.

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CHAPTER 7

PROGNOSIS IN UNEXPLAINED RECURRENT PREGNANCY LOSS: A SYSTEMATIC REVIEW AND QUALITY ASSESSMENT OF CURRENT CLINICAL PREDICTION MODELS

A. Youssef, M.L.P. van der Hoorn, J.M.M. van Lith, R. van Eekelen, N.A. du Fossé and E.E.L.O. Lashley

F&S Reviews, 2022

ABSTRACT

OBJECTIVE

To identify models predicting live birth or ongoing pregnancy in couples with unexplained recurrent pregnancy loss (RPL) and evaluate the risk of bias, performance, generalizability, and applicability of these models.

EVIDENCE REVIEW

A systematic literature search was performed in PubMed, Embase, Web of Science, and Cochrane Library until December 2020. Studies were eligible for inclusion if they were original studies predicting pregnancy outcome in patients with unexplained RPL and presented a tool that allowed for individual predictions. The risk of bias and applicability of the studies were assessed using the Prediction model Risk of Bias Assessment Tool. The Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis statement was used to assess reporting quality.

RESULTS

The search yielded 1,170 unique articles that were screened on the basis of the title and abstract. Seven studies were included: 1 prospective cohort study and 6 retrospective cohort studies. The recommended steps for the development of a prediction model were not followed by any of the studies, although 6 were published before the Prediction model Risk of Bias Assessment Tool and Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis guidelines. The included studies had a high risk of bias and were not externally validated.

CONCLUSION

International guidelines recommend supportive care programs with prognostic counselling for couples with unexplained RPL. This information manages the expectations of couples and improves their ability to make an informed decision regarding further pregnancy attempts. On the basis of the results of this study, we cannot recommend the use of any of the studied prediction models in clinical practice to prevent overestimation of chances and false belief.

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as the loss of two or more conceptions (1, 2). This condition affects 1-3% of all fertile couples (3, 4). RPL is a highly heterogeneous condition with multiple known maternal risk factors, varying from auto-immune diseases, thrombophilia and structural uterine abnormalities to advanced maternal age and maternal smoking and alcohol consumption. A paternal role (age, lifestyle factors and sperm abnormalities) is increasingly being recognized (5-7). Lastly, both parents can contribute to the risk of pregnancy losses by balanced chromosomal translocations (8-10).

Despite extensive diagnostic investigations offered to couples with RPL, a cause is identified in only 25-50% of couples (11-13). Limited understanding of mechanisms underlying unexplained RPL leads to a lack of effective treatment options. In the absence of evidence-based treatment, it is recommended by current international guidelines to offer supportive care programs for couples with RPL (14, 15). An essential part of supportive care is the counselling on prognosis and live birth rate of subsequent pregnancies. This information manages the expectations of the couple and improves their ability to make an informed decision regarding further pregnancy attempts. Various prediction models provide an estimate of subsequent chance of live birth in couples with RPL (16-18). Before they can be used in daily practice, however, prediction models should undergo accurate development, validation and impact assessment (19).

In 2015, a guideline for reporting prediction models was published and is advised to use in developing new models (20, 21). This Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) statement addresses the reporting of prediction model studies aimed at developing or validating one or more prediction models. It includes a 22-item checklist to consider when designing, conducting or analysing a prediction model. Currently this checklist is recommended by editors when submitting prediction model studies. Important items in this checklist include the quality, performance, generalizability and applicability of the prediction model. Furthermore, the PROBAST tool (Prediction model Risk of Bias Assessment tool) was developed in line with the TRIPOD

statement to assess the risk of bias of studies included in a systematic review (22, 23).

This systematic review aimed to identify all available predicting live birth or ongoing pregnancy in couples with unexplained RPL and evaluate the risk of bias, performance, generalizability, and applicability of these models according to the TRIPOD statement and PROBAST.

METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement guidelines were used in conducting this systematic review (24).

SEARCH STRATEGY AND STUDY SELECTION

A systematic search in PubMed, Embase, Web of Science and Cochrane library was performed on April 16, 2020. The following Medical Subject Headings and free text terms were used in the strategy as shown in Appendix 1 (available online): unexplained RPL; prediction model; and pregnancy outcome. The Reference lists of identified articles were manually searched for additional relevant references.

Two researchers (A.Y. and N.F.) and one librarian performed the literature search. After exporting the results of the search to a citation manager (EndNote) and removing duplicates, the articles were screened by two researchers (A.Y. and N.F.). The first stage consisted of the screening of titles and abstracts, based on the abstract describing pregnancy outcomes in couples with unexplained RPL. In the second stage, the manuscripts identified in the initial screening were read in detail. Any discordance in the selection of studies was resolved by consensus. The opinion of a third observer (E.L.) was obtained in case no agreement was obtained.

ELIGIBILITY CRITERIA

We selected studies that reported on a model to predict pregnancy outcome (ongoing pregnancy and/or live birth according to definition as used by the various authors) in patients with unexplained RPL. These pregnancies could be conceived spontaneously or by Assisted Reproductive Technology (ART). Prediction model development, validation or a combination of both could be included in this study. Prediction models could be included regardless of

when they are intended to be used (before natural conception, pre-ART or post-ART) and regardless of the timeframe in which they predict the outcome. We used English language as search limit. Exclusion criteria were: (systematic) reviews; case reports; guidelines; letters; and original articles that only describe pregnancy outcomes (without providing or developing a prediction model) in couples with RPL. In the second stage, only articles that described a prognostic model that could be used to estimate the prognosis in unexplained RPL couples were included.

ASSESSMENT OF REPORTING QUALITY

The assessment of the quality of reporting in the prediction models was performed according to the TRIPOD-statement (20). This statement provides a checklist for the development and/or validation of prediction models, consisting of 22 items and 15 subitems. The items consider the reporting of title/abstract, introduction, methods, results, discussion and other information. Each item was analysed for each study by two researchers (A.Y. and N.F.), and was answered with "Yes" if the study reported the item sufficiently, and with "No" if not. A third observer discussed any discordances in scoring the checklist (E.L.). A quality of reporting score was calculated according to the number of items that were met sufficiently. Although this score is not a validated measure of prediction model assessment, other studies have used it to summarize the quality of reporting (25-27). The TRIPOD score will therefore be primarily used here to provide a summary of the reporting quality of included studies. A narrative TRIPOD assessment will be included as supplementary material to support this analysis.

ASSESSMENT OF STUDY QUALITY

Similarly, risk of bias and applicability of the included studies in this review were assessed by two researchers (A.Y. and N.F.), using the PROBAST tool. This tool was developed based on the TRIPOD reporting guideline, and includes four domains containing 20 signalling questions for the risk of bias assessment: participants, predictors, outcome and analysis. For each domain, risk of bias was assessed separately, after which an overall estimation of risk of bias could be made. Studies were considered to be of low risk of bias if they were cohort studies with a sufficient number of participants (defined in PROBAST tool as Events Per Variable (EPV) ≥20), if

predictors were consistently defined and measured, if continuous variables were not categorized, if missing data were handled appropriately, if predictors in the final model were not included based on univariable analysis, and if model performance was assessed appropriately, followed by accounting for model overfitting (model corresponds too closely to original data) and optimism. For each study, an overall assessment of risk of bias and applicability was performed.

We performed a retrospective sample size calculation in case it was not reported, using the method as provided by Riley et al. for the calculation of a sample size in clinical prediction models, which is generally preferred over the Events Per Variable rule (28).

DATA EXTRACTION

Two reviewers (A.Y. and E.L.) extracted data from all selected articles guided by the CHecklist for critical Appraisal and data extraction for systematic Reviews of prediction Modelling Studies (CHARMS) (29). Data on study design, participants, predictors and outcome, model development methods, model performance and validation statistics, and clinical application were extracted.

MODEL PERFORMANCE

We evaluated model performance by their reported discrimination and calibration. Discrimination refers to the ability of a model to distinguish between patients who experienced the outcome and those who did not. The most widely used measure of discrimination is the concordance statistic (c-statistic), which is equal to the area under the receiver operating characteristic curve (ROC). The c-statistic is the chance that in a pair of two patients of which one had the event and one did not, the prediction model will assign a higher probability to a patient that had the event. A c-statistic of 1 means that the model always assigns the higher probability to the patient with the event whereas a c-statistic of 0.5 means that the model does not discriminate better between patients than a random guess. The second part of performance testing is calibration, which is the agreement between probabilities of grouped patients and the observed outcome frequencies. This can be portrayed in a calibration plot, which plots the observed outcome frequencies against the predicted probabilities.

RISK SCENARIOS FOR THREE HYPOTHETICAL PATIENTS

To compare the predictions across the included models, three scenarios of hypothetical patients with different characteristic were created that represent a low-, moderate- and high-risk patient. Each hypothetical patient scenario contains values for all the predictors used in the different prognostic tools. The main goal of these scenarios is to illustrate the variation in predictions across the included models but also to illustrate which model uses what predictor variables.

RESULTS

STUDY SELECTION

The study selection details are shown in the Preferred Reporting Items for Systematic Reviews and Meta-analyses flowchart in Figure 1. The main search identified 1,968 potentially relevant studies. After removing 798 duplicates, 1,170 articles remained for the first screening stage. During this screening stage, titles and abstracts were screened, after which 1,157 were excluded, whereas the remaining 13 articles were selected for second-stage screening, which comprised of full-text assessment. Six of these articles were excluded from final selection for reasons shown in Figure 1. Finally, the remaining 7 articles were included in this review (16, 18, 30-34).

STUDY CHARACTERISTICS

The key characteristics of all included studies are summarized in Table 1. Four studies were published before the year 2000 (16, 30-32) and three thereafter (18, 33, 34). All included studies were cohort studies; six were retrospective cohorts (18, 30-34) and one was a prospective cohort (16). Six single-centre studies took place in Australia (31), the United Kingdom (16, 32), Japan (33), Israel (34) and Denmark (18), and one multi-centre study did not document the locations of participating centres (30). All studies were hospital-based. The number of participants varied from 165 (31) to 1250 (33). Four studies described treatment details of their cohort (18, 30, 31, 33).

Table 1 | Summary of the key elements of the included studies

Author, year, country	Study period	Study design	Study size	Definition of RPL	Definition of pregnancy loss	Definition of outcome	Definition of unexplained	Peridctors in final model	Form of final prediction model
Cauchi 1991 Australia	?	Retrospective study consisting of 2 cohorts from different studies	165	Three or more consecutive pregnancy losses	Not mentioned	Not mentioned	Unknown, however inclusion of people who received leukocyte immunotherapy suggests that that is the unexplained RPL	Abortion times years index	Success rate formula
Quenby 1993 UK	1989 – 1992	Retrospective cohort	203	Two or more pregnancy losses	Loss of pregnancy before 14 weeks or before 22 weeks in case of previous second-trimester loss	Ongoing pregnancy beyond 14 weeks or beyond 22 weeks in case of previous second-trimester loss	Oligomenorrhea, antiphospholipid syndrome, auto antibodies, chromosomal abnormalities, viral antibodies, hormonal profiling, cervical swabs for infections, uterine	Age, menstrual cycle, previous live birth, number of previous pregnancy losses, anticardiolipin antibodies	Success rate formula
Cauchi 1995 Unknown	?	Retrospective cohort study	777	Three or more consecutive pregnancy losses by the same partner	Loss before 20 weeks gestation	Pregnancy continuing beyond 20 weeks gestation	Anatomical uterine disorders, normally ovulating, chromosome analysis, anticardiolipin antibodies and lupus anticoagulant	Abortion history, subfertility index (FI30)	Probability graph obtained from logistic regression formula (formula not mentioned)
Brigham 1999 UK	?	Prospective longitudinal observational study	226	Three or more consecutive pregnancy losses (due to patient demand, sometimes two pregnancy losses)	Loss of pregnancy before 24 weeks gestation	A survival beyond 24 weeks gestation	Antiphospholipid syndrome, oligomenorrhoea, cervical weakness, parental chromosome abnormalities. (Seems like not all associated RPL factors are mentioned)	Age, number of previous first trimester pregnancy losses	Kaplan-Meier survival curve for time-dependent pregnancy success and logistic regression formula

Author, year, country	Study period	Study design	Study size	Definition of RPL	Definition of pregnancy loss	Definition of outcome	Definition of unexplained	Peridctors in final model	Form of final prediction model
Sugiura-Ogasawara 2009 Japan	1990-2007	Retrospective cohort	1250	Two or more consecutive pregnancy losses	Not mentioned	Not mentioned	Exclusion of congenital uterine anomalies, chromosome abnormalities and persistent antiphospholipids, hyperthyroidism, diabetes mellitus and hyperprolactinemia	Age, number of previous pregnancy losses	Calculation of live birth rate in subsequent pregnancy and cumulative pregnancy using logistic regression formula
Lund 2012 Denmark	1990-2007	Retrospective cohort	987	Three or more consecutive pregnancy losses	Early miscarriage (<13 6/7 weeks) and late miscarriage (14-21 6/7 weeks)	After 22 weeks gestation	Nothing mentioned, unknown whether study group is unexplained	Age, number of pregnancy losses before first consultation	Kaplan-Meier curves for survival and cox proportional hazard ratios
Bashiri 2020 Israel	1990-2007	Retrospective cohort study	675	Two or more pregnancy losses	Not mentioned	Live birth of a baby over 24 weeks of gestation	Uterine malformations, chromosomal abnormalities, hypercoagulability or endocrine pathologies	Primary vs secondary RPL, number of pregnancy losses, negative RPL workup and patient age	Live birth rate according to total scored points

?: Unknown; FI30: Fertility Index truncated at 30 years; RPL: recurrent pregnancy loss; wk: week

DEFINITION OF CASES AND OUTCOME

Three of the included studies defined RPL as the loss of two pregnancies (32-34). One study restricted the losses to have taken place consecutively (33). The remaining four studies (16, 18, 30, 31) defined RPL as three or more consecutive pregnancy losses. The selected studies in this review defined pregnancy loss by different gestational ages, ranging from <20 weeks gestation (30), <22 weeks gestation (16, 27) to <24 weeks gestation (16, 34). Two studies did not mention gestational age in their definition of pregnancy loss (31, 33). With regard to ruling out causes of RPL, two studies did not mention any investigations or other methods to describe an unexplained RPL population (18, 31). The remaining studies (16, 30, 32-34) defined their unexplained population through excluding different causes of RPL (Table 1). The outcome (i.e., live birth or ongoing pregnancy, from here on called pregnancy success) was defined in five studies (Table 1) (16, 18, 30, 32, 34).

PREDICTORS

The age and number of previous pregnancy losses were used as predictors in three studies (16, 18, 33). One study used an "abortion times years index", which was defined as the number of pregnancy losses multiplied by the number of years over which they occurred (31). Another study by the same author used "abortion history", defined as the time in years between the first and last pregnancy loss prior to assessment, and "subfertility index truncated at 30 years of age", defined as the product of number of pregnancy losses and the abortion history (30). Age, menstrual cycle (regular vs oligomenorrhea), previous live birth, number of previous pregnancy losses and the presence of anticardiolipin antibodies were used as predictors in the study by Quenby et al. (32). Bashiri's study included primary versus secondary pregnancy loss, number of pregnancy losses, negative RPL workup and maternal age as predictors (34). The selection of predictors was only discussed in two studies, where predictors were selected based on significance in univariable analysis (31, 34).

SAMPLE SIZE AND MODELLING METHOD

The median sample size of the included studies was 675 (range 165 – 1250). None of the studies performed a sample size calculation. Approximating

that the outcome pregnancy loss occurs in 35% (Live birth occurs in approximately 65%, lowest percentage should be selected), and taking into account the number of variables included in the prediction models, the following sample sizes are required (actual sample size between parentheses): Cauchi et al. (1991); 228 (n=165), Quenby et al.; 543 (n=203), Cauchi et al. (1995); 228 (n=777), Brigham et al.; 350 (n=226), Sugiura-Ogasawara et al.; 350 (n=1250), Lund et al.; 350 (n=987), and Bashiri et al.; 350 (n=675).

Most studies performed logistic regression for the development of the prediction model (16, 30, 31, 33, 34), and one study performed a Cox regression analysis (18). The final study performed a modified regression comparing all combinations of two predictors, resulting in a model with weights per predictor. The weights resemble estimated coefficients in logistic regression (32).

PERFORMANCE, GENERALIZABILITY AND APPLICABILITY

Performance measures were only provided in three studies (32-34). These three studies only reported discrimination, no calibration was reported in any of the studies. One study only reported the ROC-curve without the c-statistic (32). The remaining two studies reported a c-statistic of 0.642 (no confidence interval (CI) provided) (33) and 0.62 (95% CI: 0.57–0.66) (34). Four out of seven studies included the full prediction model, offering a regression formula (16, 30, 33) or baseline hazard (18). Two studies presented the prediction model in a graph (31, 34), and one study presented the prediction model in a table (32). No studies presented their prediction models as an online calculation tool to be used by health professionals and patients. None of the studies were externally validated.

Three hypothetical risk scenarios were created to compare predictions across the included studies. The different predictors used with assigned values are summarized in Table 2. Figure 2 and Supplemental Table 1 (available online) show the probabilities of a successful pregnancy after RPL and portray the widespread variation in predictions across included models.

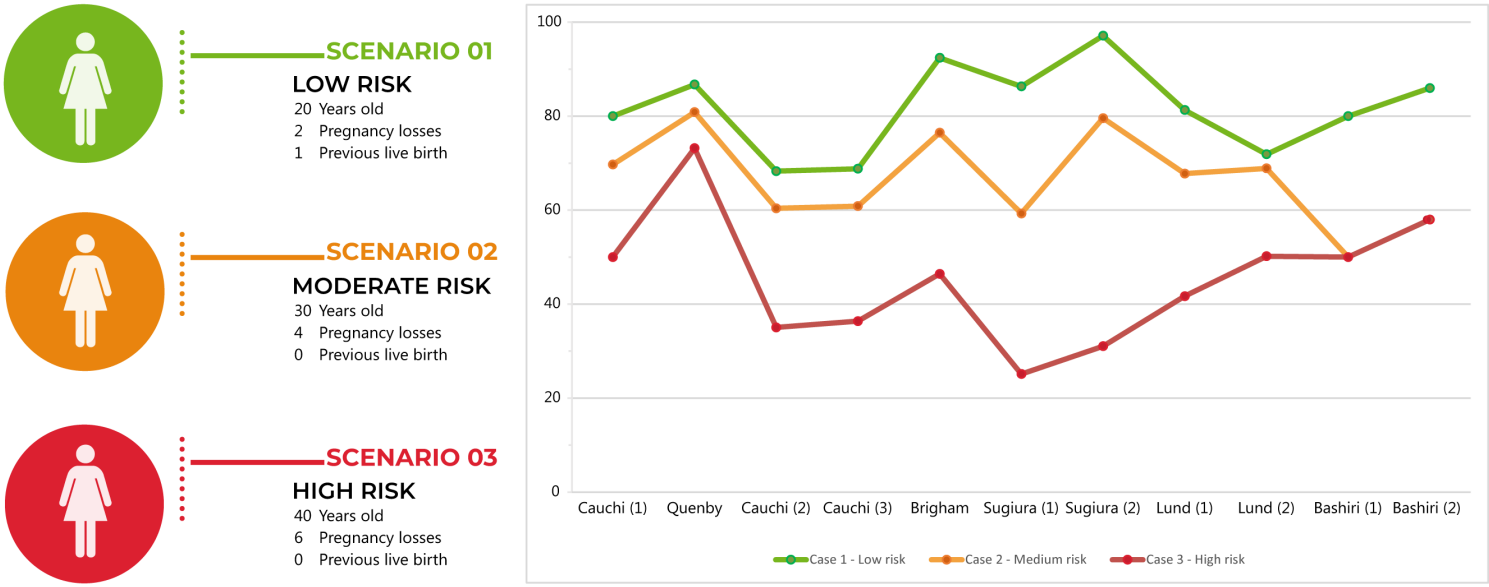

Figure 2. Illustration of risk scenarios for 3 hypothetical patients with predictors used in calculating pregnancy success chance on the left. On the right, a graph showing the predicted probabilities for each risk scenario per study. The y-axis portrays the probabilities in percentages, and the x-axis portrays the included prediction models. The green dots indicate the probability for the low-risk scenario as calculated by the corresponding prediction model on the x-axis, the orange dots indicate the moderate-risk scenario, and the red dots indicate the high-risk scenario. Cauchi [1] is the first of the 2 studies (31) of the investigator, Cauchi [2] and [3] are the second (30). Cauchi [2] uses center and abortion history as predictor, and Cauchi [3] uses center and Fertility Index truncated at 30 years (FI30) as predictor. Sugiura [1] predicts the success rate in the subsequent pregnancy, whereas Sugiura [2] predicts the cumulative pregnancy success rate (33). Lund portrays 1 Kaplan-Meier curve for age as predictor [1] and another Kaplan-Meier curve for the preceding number of pregnancy losses as predictor [2] (18). Finally, Bashiri [1] predicts the pregnancy success chance before investigations are performed, whereas Bashiri [2] does so after investigations are performed (34).

Table 2 | Hypothetical risk scenarios with selected values for all predictors			
Predictors	**Low risk**	**Moderate risk**	**High risk**
Age	20	30	40
Pregnancy losses	2	4	6
Abortion x years index	3	10	30
Previous live birth	Yes	No	No
FI30	3	10	30
Abortion history	1	3	9
Primary vs. secondary RPL	Yes	No	No

FI30: Fertility Index truncated at 30 years; RPL: recurrent pregnancy loss

A RISK OF BIAS AND QUALITY OF REPORTING

Overall, all of the included studies were at high risk of bias. The domains of the PROBAST tool are: as follows: selection of participants, predictors and their assessment, outcome and its determination and the analysis. One study scored high risk of bias in the first domain (selection) (31), three scored low risk of bias (18, 33, 34) and three scored unclear (16, 30, 32). In the second domain (predictors), three out of seven scored low (14, 26, 27) whereas the rest scored unclear (25, 26, 28, 29). In the third domain (outcome), all seven studies scored unclear. For the fourth domain (analysis), all included studies scored high risk of bias (Table 3). The main reason for the overall high risk of bias classification arises from the fourth domain on analysis, where none of the studies properly evaluated performance, and thus overfitting and optimism in model performance were not accounted for. Supplemental Table 2 (available online) shows the scores for each item per included study.

Regarding quality of reporting, the TRIPOD statement checklist was scored for each included study (Supplemental Table 3). Appendix 2 contains a narrative TRIPOD assessment of all included prediction models. As all studies were considered development studies, 9 subitems of the TRIPOD were non applicable. Thus, the final quality of reporting score was calculated as follows: score=items met/28 × 100%. The percentage of TRIPOD checklist items met for each of the seven included studies is given in Table 4 and ranged from 23 to 65% (18, 31).

Table 3 | Tabular presentation for the RPOBAST results of all included studies

	ROB				Applicability			Overall	
Study	Participants	Predictors	Outcome	Analysis	Participants	Predictors	Outcome	ROB	Applicability
1	-	?	+	-	?	+	?	-	?
2	?	+	+	-	+	+	+	-	+
3	?	?	+	-	+	+	+	-	+
4	?	+	+	-	+	+	+	-	+
5	+	?	+	-	+	+	?	-	?
6	+	+	+	-	-	+	+	-	-
7	+	?	+	-	+	+	+	-	+

+ indicates a low risk of bias/low concern regarding applicability; - indicates a high risk of bias/high concern regarding applicability; and ? indicates unclear ROB/unclear concern regarding applicability.
1, Cauchi et al. (31); 2, Quenby et al. (32); 3, Cauchi et al. (30); 4, Brigham et al. (16); 5, Sugiura-Ogasawara et al. (33); 6, Lund et al. (18); and 7, Bashiri et al. (34).
PROBAST: Prediction model Risk Of Bias ASsessment Tool; ROB: risk of bias.

Table 4 | TRIPOD score based on the checklist item reporting per included study

Study	Items met	Items not met	Score
Cauchi et al. (31)	6	22	21%
Quenby et al. (32)	14	14	50%
Cauchi et al. (30)	10	18	36%
Brigham et al. (16)	14	14	50%
Sugiura-Ogasawara et al. (33)	19	9	68%
Lund et al. (18)	20	8	71%
Bashiri et al. (34)	17	11	61%

Appendix 1 contains narrative TRIPOD assessment of the included studies

DISCUSSION

In this systematic review, seven models for the prediction of pregnancy success in couples with unexplained RPL were compared and assessed according to the TRIPOD statement and PROBAST risk of bias tool.

Our review showed that none of the included studies followed the recommended steps for model development, such as internal validation via calibration, sample size calculation and using shrinkage factors for the prevention of overfitting (35, 36). While none of the studies performed a sample size calculation, retrospective calculation showed that three studies were too small for the number of parameters fitted, indicating overfitting. With respect to the preferred prediction models by the ESHRE (17), Brigham et al. (16) and Lund et al. (16, 18), we estimated that the study of Brigham et al. could have suffered from overfitting whereas the study of Lund et al. was probably sufficiently large for their relatively simple model. The continuous variables age and number of pregnancy losses were often categorized, which could lead to a loss of accuracy as patient information is discarded by considering patients of different ages and patients with different obstetrical history as identical (35). The categorization of continuous variables is associated with various problems, including the aforementioned assumption of homogeneity withing groups, but could also result in associations between categories that are not necessarily true (5, 37).

Following the TRIPOD statement, it is important to know the reason of missing data of the included studies. It is common to have missing data to some extent, for instance due to typos or administrative mistakes. However, systematic reasons for missing data may lead to biased predictions due to selection. Cohorts included in this review were generally complete cases cohorts but it was not reported how much missing data was present before continuing with the complete cases. Therefore, whether the data was missing (completely) at random or not at random, or the amount of influence on the predictions, remains unclear.

Given the fact that in the past many experimental treatments have been given in unexplained RPL populations, it is important to describe such treatments as they may affect outcomes. Only four of the seven included studies in this review described treatments given to participants (18, 30, 31, 33). Treatments described in these studies include paternal mononuclear cell immunization, low dose aspirin and intravenous immunoglobulin, which have all been shown ineffective in improving live birth rates in couples with RPL (38-40). It is therefore not likely that treatments given in other studies for unexplained RPL have impacted prognosis. For future studies, experimental treatments could however be included as predictor because of the uncertainty of their prognostic effect.

The predictive performance of prediction models is described using discrimination and calibration (36, 41). Three studies reported discrimination (32-34), and only two reported the c-statistic: In the study by Sugiura-Ogasawara et al., the c-statistic was 0.642 for subsequent pregnancy risk calculation and the c-statistic in the study by Bashiri et al. was 0.62, which are both considered as moderate. This is not surprising given the few predictors used in the models in this review and given how little we understand of RPL. None of the other studies provided a measure of discrimination. Furthermore, none of the studies performed calibration of their models, which is arguably more important than assessing discrimination (42).

The aforementioned limitations of the studies can be illustrated using different clinical scenarios. With the help of three hypothetical scenarios, we illustrated the difference between the models by providing their predictions in three categories: a low risk, moderate risk and high risk

unexplained RPL patient. This is merely an illustration of varying predictions, partly explained by the difference in predictors and inclusion/exclusion criteria. Figure 2 should not be interpreted as preferring one model over the other. A direct comparison of calculated rates between studies is best performed via external validation.

An important drawback of using the RPL prediction models in clinical practice is the lack of any validation. Internally validating a prediction model is important, as it can be expected to perform well because the model was designed to fit the development data (36). This leaves room for overfitting, meaning that the model would become less accurate when tested in new but similar individuals. Internal validation is performed in the original dataset, and bootstrapping is the preferred technique (36). External validation on the other hand is conducted in a separate, unrelated cohort and is needed to confirm that the developed model also predicts well in similar but unrelated individuals (43). This resembles the usage of the model in new, future patients and is thus a crucial step for prediction model implementation (43).

The application approach of these models is important for clinical practice: a user-friendly interface, nomogram or calculator facilitate both patients and clinicians to quickly and reliable use the model. The included studies most often used a logistic regression formula as a final model, which on itself is not a user-friendly way but which can easily be implemented elsewhere. Two studies used graphs for estimation of risk, which may look more user-friendly but leaves room for error in reading the predictions (18, 31). One study portrayed probabilities in a graph according to total scored points and, in addition, specified the exact probability for each amount of points scored (34).

Based on their calculated TRIPOD-scores, the included studies can be arbitrarily divided in three groups: Low (<40%) (16, 31), average (around 50%) (16, 32, 34) and above average (>60%) (18, 33). This division in quality of reporting is correlated with the period of publication, as more recent studies score higher. A possible explanation is that the TRIPOD-statement was published in 2015, however literature before this statement has been widely available as guidance for development and validation of

prediction models (20). The results of this review however could serve as a call for future prediction studies to follow the TRIPOD-statement.

As performance measures were not mentioned in all studies, and as external validation has not been performed in any of the studies, all studies score high risk of bias in the PROBAST tool, and we could not identify studies that we recommend for predicting pregnancy success in couples with unexplained RPL.

Counselling with prediction models is of utmost importance in couples with unexplained RPL. In the absence of an underlying cause in these couples with the lack of a treatment trajectory, estimation of their chances for future successful pregnancies helps with expectation management and might help in preparing alternatives to start a family. The prediction models cater to different, heterogeneous RPL populations, which makes it difficult to apply in any other setting or to a contemporary RPL population. Two models are implicated in the internationally widely used ESHRE RPL guideline (16, 18), of which one is not a multivariable prediction model and is not suitable for the prediction of future live birth, as stated by the authors themselves (18). The other model was neither internally nor externally validated and is outdated with respect to definition of the study population (16). Following the results of this review, unfortunately we conclude that using these models, clinicians provide counselling that is at best inaccurate and at worst, incorrect.

A limitation of this study follows from the observation that none of these studies specifically describe themselves as prediction models. Possibly, more prediction studies exist that were not included in this review. However, our broad search was conducted in different databases and independently performed by two researchers, therefore we believe that the probability of missing a prediction study in our inclusion is low. Next, the TRIPOD-score is not a validated score, but a reflection of the TRIPOD-checklist. This score has however been used in different studies to easily summarize the quality of reporting (25-27).

To our knowledge, this is the first systematic review assessing literature that describes prognostic tools of pregnancy success in couples with unexplained RPL. The methodological evaluation of existing prediction models according to the CHARMS checklist, the TRIPOD statement and

PROBAST tool is one of the main strengths of this review. Thereby, we evaluated RPL studies on methodology, risk of bias in their methodology, and on quality of reporting. Another major strength is that the results of this review can be a basis for the current best-available evidence to be used in counselling as well as provide pointers for future research.

CONCLUSION

On the basis of our results, we cannot recommend any prediction model in clinical practice. We suggest that external validation of the existing models needs to be conducted. The prediction models by Sugiura-Ogasawara et al. and Bashiri et al. seem most appropriate for this external validation, according to the low score in three PROBAST domains (33, 34). On the basis of these results, we can assess whether these models perform reasonably or if a new approach is preferred altogether.

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APPENDIX 1

Search string, performed on 10-11-2020. Available online at: https://www.sciencedirect.com/science/article/pii/S2666571922000020

APPENDIX 2

NARRATIVE TRIPOD ASSESSMENT

As mentioned, the TRIPOD statement includes a 22-item checklist, divided in six sections. These sections and the corresponding items will be explored in this section. Appendix 2 shows an overview of how the included studies scored per item of the TRIPOD checklist.

TITLE AND ABSTRACT

All studies except one (31) include an abstract that summarizes the context, setting, method, results and conclusion. The six remaining studies with a clear structured abstract however do not discuss the statistical method used for the main objective (16, 18, 30, 32-34).

INTRODUCTION

Five out of seven studies explain the medical context, with some referring to other prediction models. The two studies by Cauchi et al. provide explanation on RPL, but not on why they developed a prediction model (30, 31). Only four of the included studies specify the objectives (16, 18, 33, 34). What stands out is that none of the studies explicitly write that they are developing a model, although the outcome is a model of some sort that provides the estimation of pregnancy success. The provided objectives are "to predict future pregnancy success based on gestational age, maternal age and miscarriage history" (16), "to assess live birth rate on a prospective basis, according to maternal age and previous number of miscarriages" (33), "to establish a method of estimating the proportion of women with a subsequent live birth after a well-defined time period" (18) and "to create a prognostic tool to predict the chance of a live birth in patients with recurrent pregnancy loss using easily acquired baseline variables" (34).

METHODS

With the exception of one study (30), all studies described study design and source of data. Cauchi et al. stated that seven centres submitted 777 patients, without describing from what source these patients were included

(30). Three studies did not specify key study dates (16, 30, 31), i.e., start of the study, end of the study and end of the follow-up period. The two studies by Cauchi et al. did not include key elements of study setting (30, 31). The eldest study of the two combined the results of two other studies referring to only one of the two studies, the setting remains unknown. The second study by Cauchi et al. was a multicentre study, with unknown locations and unknown care settings.

Eligibility criteria were included in four out of seven studies, and differed from one another (16, 18, 30, 33). As mentioned, the eldest study by Cauchi et al. included two study populations and eligibility criteria are unknown (31). Quenby et al. stated that patients were referred to the clinic by their general practitioner, but no details were provided on requirements of referral (32). Bashiri et al. mentions that women who were treated at the clinic were included, but did not mention the criteria couples should fulfil to visit the RPL clinic (34).

Treatment details were given in four of seven articles (18, 30, 31, 33). This is important, given the fact that in the past many experimental treatments have been given in unexplained RPL populations. Quenby et al., Brigham et al. and Bashiri et al. did not mention anything about treatments received by participants (16, 32, 34).

The outcome in all studies was to predict successful pregnancy. One study by Cauchi et al. did not explicitly mention what the outcome was that was predicted in the model, i.e., what exactly a successful pregnancy was (31). The paper mentioned that "primary aborters" are those who did not have a pregnancy continuing past 20 weeks gestation before, but nowhere was it mentioned that this is regarded as a live birth. The six other studies did define outcome (16, 18, 30-32, 34), although one study only mentioned live birth as outcome without defining live birth (33). Other studies provided a definition regarding gestational age from which pregnancy is called ongoing or live birth. Most articles provided a general definition for predictors; however, three studies did not define the predictor miscarriage (limit for gestational age) (31, 33, 34). Two studies described predictor selection, which was based on a univariate analysis (31, 34).

Sample size calculation or explanation was not included in any of the studies and none of them described how missing data were handled. The

same applies to the way predictors were handled in the analysis. None of the studies mentioned whether predictors were categorized, and if so, why specific cut-off points were chosen. Some tables provided insight in how predictors were handled, however none of the studies explicitly explained how they came to using these predictors. All studies described the type of model they chose, but none of them specified measures to assess the performance of the chosen model after developing it. One study mentioned internal validation; however the description of this process seems to be measuring the performance of the study, and not internal validation (34).

RESULTS

Only one study did not describe the flow of participants throughout the study (31), as the participants of that study originate from two other studies. Although the other six studies did describe the flow of participants throughout the study (16, 18, 30, 32-34), only the study by Lund et al. provided a flow diagram (18). As most of the studies used first pregnancy after diagnosis as outcome measure, median follow up time was not summarized. Baseline characteristics were not mentioned in the study by Cauchi et al.(31). Table 1 already consisted of results, and moreover showed that there seems to be missing data, which was not mentioned in text. The study by Quenby et al. showed baseline characteristics for those of the population who conceived successfully, but not for the population that did not conceive (32). Although the second study by Cauchi et al. did show baseline characteristics, it lacked information on missing data regarding the outcome, as seen in the result tables which do not add up to the total study population (30).

In that second study by Cauchi et al, it was also not clear which participants were included in the final analysis that resulted in the regression model for the prediction of pregnancy success (30). The other studies did report the number of participants and outcome event in each analysis (16, 18, 31-34). Six studies developed a multivariable model for the prediction of pregnancy success (16, 30-34). Only two studies reported the unadjusted association of the included prediction parameters (18, 33). In Sugiura-Ogasawara's study this was mentioned in the results section for age and number of previous pregnancy losses, which were finally used in the logistic regression

model (33). Lund did not develop a multivariable model, but reported the individual hazard risks for age category and miscarriage category (18).

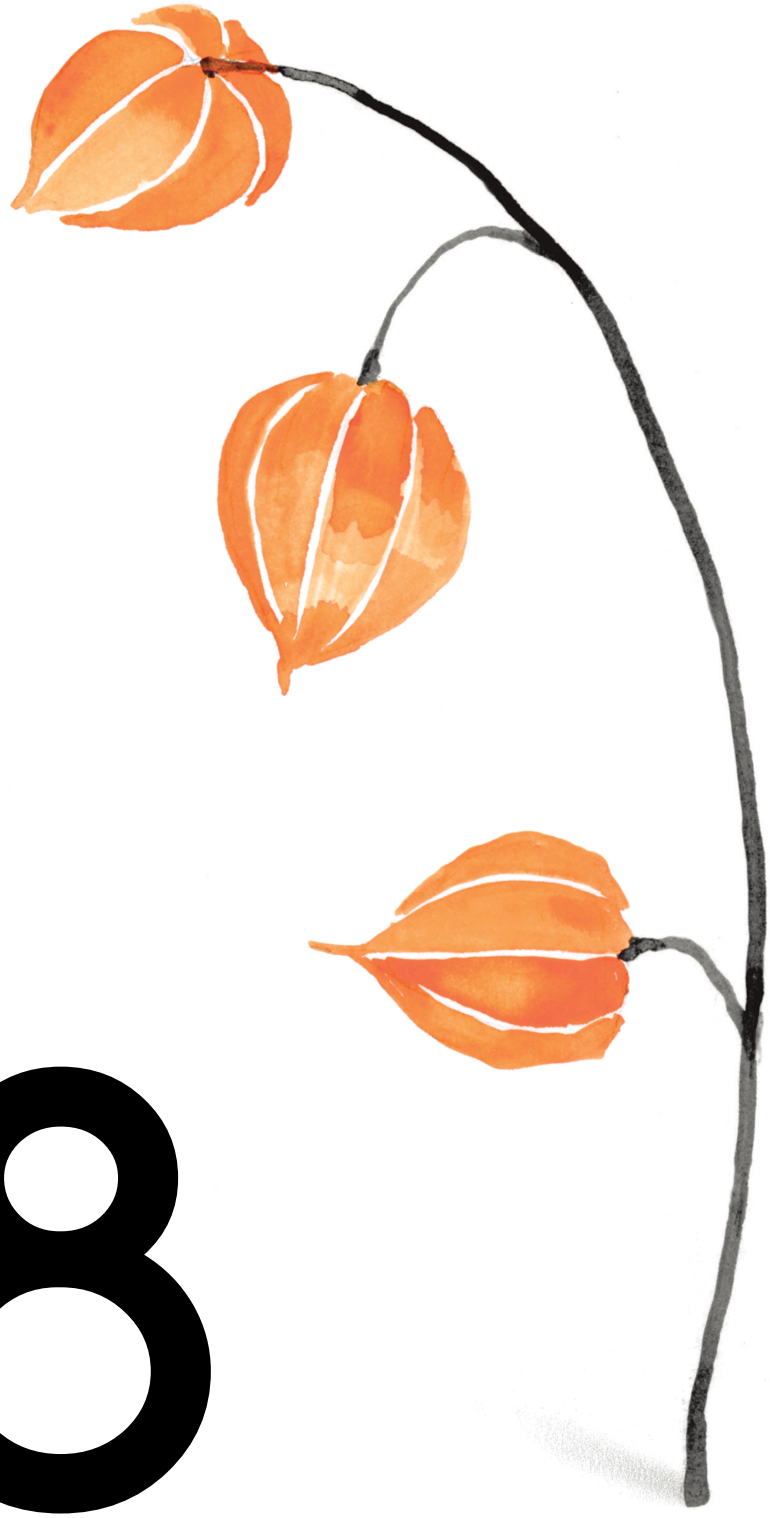
Five studies presented the full prediction model, which allowed for individual predictions. Cauchi et al. and Lund et al. however did not present a model that allowed for individual predictions (18, 31). Both presented graphs which allowed for approximation of individual risk. Furthermore, as mentioned before, Lund did not develop a multivariable model, but reported two graphs with risk estimation for two predictors (18). All studies explained the usage of the model, except for Brigham (18, 30-33). In the article of Brigham et al., the logistic regression formula was mentioned in the methods section, and nowhere was explained how to use the formula (16). In the results section, a table was presented with risk estimation for individuals with age gaps of five years. It is unknown which of the two Brigham intended to be used for individual prediction. Although the formula is most likely the one to be used, no explanation is provided.

Performance measures were only provided in three studies (32-34). Quenby et al. provided the Receiver Operator Curve (ROC) and sensitivity and specificity (32). Sugiura-Ogasawara et al. and Bashiri et al. reported ROC as well, with concordance statistic (33, 34). Although more performance measures could have been added, they still outperform the other studies, as the other four studies did not include any performance measure reporting (16, 18, 30, 31).

DISCUSSION

Only one study discussed its limitations. Lund explained that the study cannot be used for individual risk approximation, as it was not designed to do so according to the authors (18). Furthermore, Lund explained that the study could not differentiate between explained and unexplained RPL. All studies reported their interpretation of the results and compared their results to previous literature (16, 18, 30-34).

Lastly, none of the studies provided information on supplementary resources, and only three studies provided a statement on funding or conflict of interest (18, 33, 34).



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CHAPTER 8

EXTERNAL VALIDATION OF A FREQUENTLY USED PREDICTION MODEL FOR ONGOING PREGNANCY IN COUPLES WITH UNEXPLAINED RECURRENT PREGNANCY LOSS

A. Youssef, M.L.P. van der Hoorn, M. Dongen, J. Visser, K.W.M. Bloemenkamp, J.M.M. van N. van Geloven and E.E.L.O. Lashley

Human Reproduction, 2022

ABSTRACT

STUDY QUESTION

What is the predictive performance of a currently recommended prediction model in an external Dutch cohort of couples with unexplained recurrent pregnancy loss (RPL)?

SUMMARY ANSWER

The model shows poor predictive performance on a new population; it overestimates, predicts too extremely and has a poor discriminative ability.

WHAT IS KNOWN ALREADY

In 50-75% of couples with RPL, no risk factor or cause can be determined and RPL remains unexplained. Clinical management in RPL is primarily focused on providing supportive care, in which counselling on prognosis is a main pillar. A frequently used prediction model for unexplained RPL, developed by Brigham et al. in 1999, estimates the chance of a successful pregnancy based on number of previous pregnancy losses and maternal age. This prediction model has never been externally validated.

STUDY DESIGN, SIZE, DURATION

This retrospective cohort study consisted of 739 couples with unexplained RPL who visited the RPL clinic of the Leiden University Medical Centre (LUMC) between 2004 and 2019.

PARTICIPANTS/MATERIALS, SETTING, METHODS

Unexplained RPL was defined as the loss of two or more pregnancies before 24 weeks, without presence of an identifiable cause for the pregnancy losses, according to the European Society for Human Reproduction and Embryology (ESHRE) guideline. Obstetrical history and maternal age were noted at intake at the RPL clinic. The outcome of the first pregnancy after intake was documented. The performance of Brigham's model was evaluated through calibration and discrimination, in which the predicted pregnancy rates were compared to the observed pregnancy rates.

MAIN RESULTS AND THE ROLE OF CHANCE

The cohort included 739 women with a mean age of 33.1 years (±4.7 years) and with a median of three pregnancy losses at intake (range 2-10). The mean predicted pregnancy success rate was 9.8 percentage points higher in the Brigham model than the observed pregnancy success rate in the dataset (73.9% vs 64.0% (CI 95% for the 9.8% difference 6.3% – 13.3%)). Calibration showed overestimation of the model and too extreme predictions, with a negative calibration intercept of -0.46 (CI 95% -0.62 – -0.31) and a calibration slope of 0.42 (CI 95% 0.11 – 0.73). The discriminative ability of the model was very low with a concordance statistic of 0.55 (CI 95% 0.51 – 0.59). Recalibration of the Brigham model hardly improved the c-statistic (0.57; CI 95% 0.53 – 0.62)

LIMITATIONS, REASONS FOR CAUTION

This is a retrospective study in which only the first pregnancy after intake was registered. There was no time frame as inclusion criterium, which is of importance in the counselling of couples with unexplained RPL. Only cases with a known pregnancy outcome were included.

WIDER IMPLICATIONS OF THE FINDINGS

This is the first study externally validating the Brigham prognostic model that estimates the chance of a successful pregnancy in couples with unexplained RPL. The results show that the frequently used model overestimates the chances of a successful pregnancy, that predictions are too extreme on both the high and low ends, and that they are not much more discriminative than random luck. There is a need for revising the prediction model in order to estimate the chance of a successful pregnancy in couples with unexplained RPL more accurately.

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as the loss of two or more conceptions (1). This condition affects 1-3% of all fertile couples (2, 3). RPL is a highly heterogeneous condition with multiple known maternal and paternal risk factors (4-6). Despite extensive diagnostic work-ups offered to couples with RPL, an underlying risk factor may be identified in only 25-50% of couples (7, 8). Limited understanding of mechanisms underlying RPL leads to the lack of options for effective treatment. As no evidence-based therapeutic options are available for couples with RPL, clinical management is primarily focused on providing supportive care. Supportive care and intensive pregnancy surveillance in the first trimester of gestation is assumed to be of influence in the prevention of new pregnancy loss (9). An important aspect of this supportive care is counselling on the prognosis and success rate of subsequent pregnancies in couples with RPL.

Several prediction models for the estimation of the chance of live birth after RPL have been published (10-16) and various international guidelines recommend the use of different prediction models (17). The European Society of Human Reproduction and Embryology (ESHRE) RPL guideline recommends to use the prediction models of Brigham et al. or Lund et al. (hereafter called the "Brigham model" and the "Lund model") to estimate the chance of live birth in couples with unexplained RPL (1). The Brigham model has been implemented in RPL care in the Netherlands and in the United Kingdom (18, 19), while the American Society for Reproductive Medicine (ASRM) adapted the Lund model in their RPL guideline (20). The Lund model was not designed for individual risk assessment, given the descriptive scope of the study. Furthermore, the study does not discriminate between unexplained and explained RPL. Although the Brigham model and the Lund model were both reviewed with high methodological quality and both studies have consistent results, these models did not follow the nowadays recommended TRIPOD guideline in the development and reporting of a prediction model (21). This guideline provides a 22-item checklist consisting of items that assures transparent reporting, and acts as a tool for reminding authors of all necessary prediction components, such as measuring the predictive performance of the study internally and/or externally. Both models were never internally

nor externally validated, which leaves their predictive performance unknown.

As the Lund model was not intended for individual risk assessment, the aim of this study is to externally validate the Brigham model to assess its predictive performance in a Dutch cohort of couples with unexplained RPL.

METHODS

PATIENT POPULATION

We included couples with unexplained RPL who visited the clinic of the Leiden University Medical Centre (LUMC) for intake consultation between 2004 and 2019. We defined unexplained RPL as the loss of two or more pregnancies until 24 weeks, without presence of an identifiable cause for the pregnancy losses, according to the ESHRE guideline (1). The following investigations were performed to rule out factors associated with RPL: maternal testing for antiphospholid syndrome (lupus antibodies, anticardiolipin antibodies, anti-β2-glycoprotein antibodies), parental karyotyping for chromosomal abnormalities based on a priori chance (22), endocrinological factors (thyroid function and thyroid peroxidase antibody testing, random glucose level on indication (23)), and assessment of uterine cavity to rule out anatomic abnormalities. Testing for inherited thrombophilia and hyperhomocysteinemia was performed until 2018 as these were regarded associated factors for RPL. Since the publication of the ESHRE guideline in November 2017, thrombophilia and hyperhomocysteinemia testing were excluded from the RPL investigations and are only performed to rule out an increased chance of thrombotic events, as is now daily practice at our clinic. RPL couples who tested positive for either, but did not have any other associated RPL factors, were regarded as unexplained RPL in this study. After intake at the LUMC RPL clinic, intensive pregnancy surveillance in the first weeks of gestation was offered in a new pregnancy, consisting of weekly ultrasound checks performed by an easily accessible and dedicated RPL team.

DATA COLLECTION

Data collection was performed according to the Brigham model. We retrieved maternal age and number of preceding miscarriages at time of intake at the RPL clinic. The outcome of the first pregnancy after intake at

the clinic was registered. A successful outcome was regarded as ongoing pregnancy (heartbeat on ultrasound) beyond 24 weeks. Only patients with a known pregnancy outcome were considered for inclusion. Couples missing this data were assumed not to differ systematically from couples with complete data.

STATISTICAL ANALYSIS

We evaluated the predictions of the Brigham model through calibration and discrimination. Calibration examines the agreement between the predicted and observed pregnancy success rates, while discrimination refers to the ability of the model to separate women with a successful pregnancy from those without. Therefore, we calculated the percentages of a successful pregnancy according to the formula described by the Brigham model, as shown below (10).

$$\log(p/(1-p))=2.00-0.0828\ (age-32)-0.2467\ (number\ of\ pregnancy\ losses)$$

Here, p is the predicted probability of a vital pregnancy for those patients who reached pregnancy. We performed a graphical assessment of the calibration, using the val.prob.ci.2 function, obtained from the library CalibrationCurves (https://github.com/BavoDC/CalibrationCurves), of the R statistical program (version 4.0.2). This function validates predicted probabilities against binary events, computing a set of indexes and statistics.

Based on these indexes and statistics, a calibration curve is plotted, including a calibration intercept, which indicates the extent that predictions are systematically too low or too high (also called "calibration in the large"), and a calibration slope. In a perfectly calibrated model, the intercept is 0 and the slope is 1. An intercept with a negative value suggests overestimation, while an intercept with a positive value suggests underestimation. A slope < 1 suggests that the estimated chances are too extreme, while a slope > 1 suggests that the estimated risks are too moderate (24).

The discriminative ability of Brigham's model was measured using the concordance statistic (c-statistic). It gives the probability that a randomly selected patient who achieved a successful pregnancy had a higher estimated chance than a patient who did not. A value of 1 means that the

model perfectly predicts who will experience a successful pregnancy and who will not. A value of 0.5 means that the model is no better at predicting than random chance.

To see whether the Brigham model would perform better after recalibration to our validation data, we followed the methods described by Vergouwe et al. (25). Three additional logistic regression models were estimated: one updating the intercept of the model (recalibration in the large), one updating the intercept and the strength of the predictors (logistic recalibration), and model revision (estimating all model parameters anew). The performance of these updated models was assessed using the same metrics as for the original Brigham model.

SAMPLE SIZE CALCULATION

For the calculation of the required sample size for this external validation, we used the method described by Riley et al. for the calculation of a sample size in clinical prediction models (26). We indicated that we were using the same two variables as Brigham: age and number of previous first trimester pregnancy losses, both as continuous variables. A value of 0.1089 was calculated for the R2, the expected shrinkage was set to 0.9, as suggested by Riley et al. The prevalence of a pregnancy loss was expected to be 35% (27). The R package pmsampsize provided alongside the paper of Riley et al. was used for the calculation of the sample size. Each step leads to a calculated sample size, and the largest sample size is the required sample size. This resulted in a sample size of 350 couples with unexplained RPL who achieved a new pregnancy after intake at the clinic.

ETHICAL APPROVAL

Approval for this study and data collection was obtained at the Medical Research Ethics Committee of the Leiden University Medical Centre (protocols P11.196 and P19.014).

RESULTS

Between 2004 and 2019, 904 couples with unexplained RPL were registered at the LUMC RPL clinic. Of these 904 couples, 107 (11.8%) were lost to follow up, and 58 couples did not conceive a pregnancy after intake, which resulted in a group of 739 couples with a known outcome of the first pregnancy after intake at the RPL clinic. These 739 couples are included in the analysis (Figure 1).

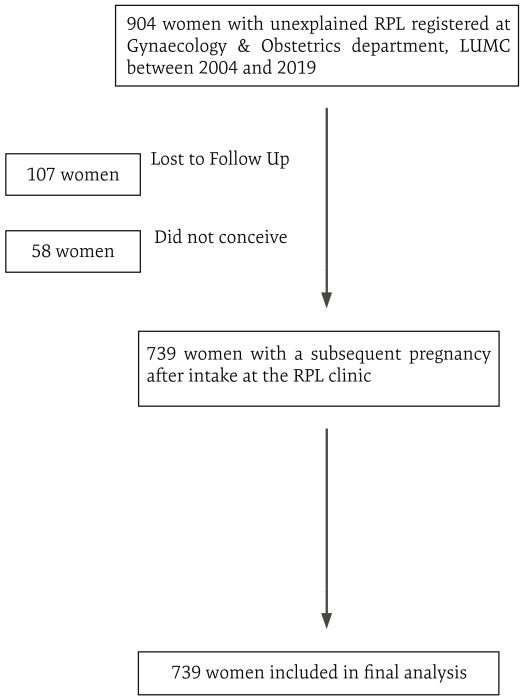

Figure 1. Flow chart of women with unexplained recurrent pregnancy loss who were considered for inclusion in external the validation.

The mean age of the women was 33.1 years (±4.7 years), with a median of three pregnancy losses at intake (range 2-10 pregnancy losses). More than half of the couples (60.5%) had not previously given birth (live births; range 0-4). The baseline characteristics of these couples are shown in Table 1. The group of patients who were lost to follow up was comparable at baseline, with a mean age of 33.6 years (±4.7 years), a median of three pregnancy losses at intake (range 2-8 pregnancy losses) and a median of zero live births (range 0-5). The first pregnancy after intake was successful in 64.1% (CI 95% 60.6% - 67.6%) of couples, defined as a heartbeat on ultrasound ≥24 weeks pregnancy. Data of first pregnancy after intake is shown in Table 2.

Table 1 | Baseline characteristics at time of intake (n=739)	
Age (years)	33.1 (±4.7)*
20-24	35 (4.7%)
25-29	140 (18.9%)
30-34	276 (37.3%)
35-39	234 (31.7%)
≥40	54 (7.3%)
Number of previous pregnancy losses (n)	3 (2-10)+
2	103 (13.9%)
3	3941 (53.3%)
4	50 (20.3%)
≥5	92 (12.4%)
Previous live birth (n)	0 (0-4)+
0	447 (60.5%)
1	236 (31.9%)
≥2	56 (7.6%)
Year of inclusion (n)	
2000-2004	50 (6.8%)
2005-2009	180 (24.4%)
2010-2014	279 (37.8%)
2015-2019	230 (31.1%)

* Mean with standard deviation between parentheses

+ Median with range between parentheses

Table 2 | Overview of outcome data in numbers (n=739)	
No pregnancy	58 (6.4%)+
Lost to follow-up	107 (11.8%)
Biochemical pregnancy	74 (10.0%)
Clinical pregnancy loss in first trimester	158 (21.4%)
Clinical pregnancy loss in second trimester	2 (0.3%)
Live birth (pregnancy ≥24 weeks gestation)	474 (64.1%)
Pregnancy loss (not further clarified	31 (4.2%)

+ Percentage calculated based on cohort population before exclusion (n=904)

We plotted the expected success probabilities of the first pregnancy after intake according to Brigham's formula against the observed rates (Figure 2). The mean predicted pregnancy success rate using the Brigham model was 9.8 percentage points higher than the observed pregnancy success rate in the dataset (73.9% vs 64.0% (CI 95% for the 9.8% difference 6.3% – 13.3%)).

Calibration in the large resulted in a statistically significant intercept of -0.46 (CI 95% -0.62 – -0.31), affirming the higher predicted success rate. The slope of the calibration curve was statistically significant at 0.42 (CI 95% 0.11 – 0.73). The c-statistic, used to describe the discriminative ability of the prediction model, was 0.55 (CI 95% 0.51 – 0.59).

Calibration in the large, logistic recalibration and model revision each led to an improvement in model fit (each Likelihood ratio test comparing against the original model P value = <0.001), thus full model revision was adopted. The revised model was estimated as follows:

log(p/(1-p))=1.53-0.01 (age-32)-0.28 (number of pregnancy losses)

However, the updated model barely improved its discriminative ability (c-statistic 0.57; CI 95% 0.53 – 0.62).

DISCUSSION

To improve counselling as part of supportive care of RPL couples, accurate predictions on pregnancy success are of utmost importance. This study is the first to externally validated the frequently used Brigham model that predicts outcome of next pregnancy in couples with unexplained RPL, as developed by Brigham et al. (10). This resulted in a calibration curve with a negative intercept, a slope smaller than 1.0 and a c-statistic of 0.55.

A calibration slope of < 1 suggests that the estimated risks are too extreme, meaning that the predicted chances are too low for older couples with a higher number of pregnancy losses and that the predicted chances are too high for younger couples with lower number of pregnancy losses. In other words, the effect of age and number of pregnancy losses is stronger in the Brigham model than in the validation dataset. The value of the c-statistic ranges from 0.5 to 1.0, with 0.5 indicating prediction based on pure chance and 1.0 indicating perfect prediction. According to our analysis, there is poor predictive performance of this model on a new population. The model overestimates, has too extreme predictions and has a poor discriminative ability.

It is already known that the accuracy of prediction models is often lower in a separate cohort (28). We tried updating the model in our new data; however, the discriminative ability did not improve and the model revision

led to re-estimation of all coefficients, which disregards information from the original dataset. Our data suggests that age and number of previous pregnancy losses alone are not able to discriminate between patients with or without a successful next pregnancy.

The ESHRE and RCOG (Royal College of Obstetricians and Gynaecologists) guidelines mention that couples with unexplained RPL have high chances of achieving a live birth in the future, using the Brigham prediction model as substantiation. In our study however, we observed that the predicted chances of the model are much higher than the actual success rate, reflected by the 9.8 percentage points difference between the mean predicted success rate and the actual live birth rate. The majority (76%) of patients in the dataset of the Brigham model had a history of three or more miscarriages, and the remaining 24% consisted of patients with two miscarriages who requested analysis for the RPL. In our dataset only 14% (103/739) of patients experienced two miscarriages, which could explain the overall lower mean chance of success.

We expected a higher age in our study population, as in general a trend of delaying motherhood is present (29). This higher age could also explain the observed difference in predicted pregnancy success. However, the mean age in our cohort (33.1 ±4.8 SD years) does not differ from the mean age in the cohort in the Brigham model (32 years), though for the latter the age range was not presented. Finally, the setting of the two cohorts could be different. Our centre is a tertiary referral centre, but also includes patients referred by primary care. The setting of Brigham's cohort is unknown.

The poor performance of the model in our cohort could also be explained by the model's development. The Brigham model was based on a prospectively collected dataset of 716 patients with RPL. However, only 325 of them were identified as having `idiopathic recurrent miscarriage' and 23 patients were lost to follow-up. A subsequent pregnancy was achieved by 226/325 (70%) patients, of which two were found to be ectopic, and two patients underwent termination of pregnancy. Thus, the model was based on only 222 patients and this small number could have resulted in overfitting of the model. This is demonstrated in the sample size calculation, that points to a total of 350 patients necessary for a model with two continuous variables. Furthermore, as no internal validation was performed during model

development to correct for the degree of overfitting (such as bootstrapping), it is evident that the performance of the model is better on its training dataset than in another or external dataset (30). In short, there was poor development of the study due to underpowering, lack of internal validation and lack of external validation.

Next, the likelihood of finding a low predictive accuracy during validation will increase if a more stringent form of validation is used (31, 32). In our study, we included patients from another geographical area and from another time period. This has influence on differences between the populations. First, the definition of RPL has significantly changed over the past 20 years (1). Women with antiphospholipid syndrome, oligomenorrhoea, cervical weakness and abnormal parental chromosome karyotype and patients with a history of second trimester loss were excluded from the dataset in the Brigham model. According to the current definition, oligomenorrhoea is not considered a factor for recurrent pregnancy losses. Furthermore, RPL nowadays includes all pregnancy losses from the time of conception until 24 weeks of gestation. Brigham et al. also excluded "those who had completed successful treatment of an abnormal finding", which is not specified any further in the study.

This study is the first to externally validate the Brigham model, a frequently used prognostic model for successful pregnancy in RPL care. With the large sample size in our study, our evaluation of the model provides precise model performance measures. We followed Brigham's research method to the best of our abilities, to ensure that the external validation was performed on equally developed models. Regarding the outcome, pregnancy success was defined as a pregnancy continuing beyond 24 weeks of gestation, rather than a live birth, which is what patients ultimately want to know. As indicated by Smith et al, there is a need for standardised and patient-central clinical outcomes in studies on pregnancy after RPL (33).

Importantly, our study only included cases with a known pregnancy outcome in the analysis. In our cohort, the main reason for unknown pregnancy outcomes, is that couples leave the clinic around the tenth week of gestation and continue their pregnancy care given by a community midwife. We assumed that missing data was unrelated to the variables involved in the analysis, and therefore did not bias the analysis. This

assumption was supported by the fact that patients with missing data were comparable in age, pregnancy losses and live births at baseline. Moreover, missing data and loss of follow up could also be explained by the inability of couples to achieving a new pregnancy, either voluntary or involuntary, and these couples would not have been included for this study.

Our study shows that the Brigham model does not perform well in a Dutch population. The poor discriminative ability of this model implies that it should not be used routinely in the counselling and prognosis on subsequent pregnancies in patients with RPL. Instead, the model should be revised in order to estimate the chance of a successful pregnancy in couples with unexplained RPL more accurately.

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9

CHAPTER 9

DEVELOPMENT OF THE OPAL PREDICTION MODEL FOR PREDICTION OF LIVE BIRTH IN COUPLES WITH RECURRENT PREGNANCY LOSS: PROTOCOL FOR A PROSPECTIVE AND RETROSPECTIVE COHORT STUDY IN THE NETHERLANDS

A. Youssef, M.L.P. van der Hoorn, R. van Eekelen, N. van Geloven, M. van Wely, M.A.J. Smits, A.G.M.G.J. Mulders, J.M.M. van Lith, M. Goddijn and E.E.L.O. Lashley

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ABSTRACT

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as the loss of two or more conceptions before 24 weeks gestation. Despite extensive diagnostic workup, in only 25%–40% an underlying cause is identified. Several factors may increase the risk for miscarriage, but the chance of a successful pregnancy is still high. Prognostic counselling plays a significant role in supportive care. The main limitation in current prediction models is the lack of a sufficiently large cohort, adjustment for relevant risk factors, and separation between cumulative live birth rate and the success chance in the next conception. In this project, we aim to make an individualised prognosis for the future chance of pregnancy success, which could lead to improved well-being and the ability managing reproductive choices.

METHODS AND ANALYSIS

In this multicentre study, we will include both a prospective and a retrospective cohort of at least 931 and 1000 couples with RPL, respectively. Couples who have visited one of three participating university hospitals in the Netherlands for intake are eligible for study participation, with a follow-up duration of 5 years. General medical and obstetric history and reports of pregnancies after the initial consultation will be collected. Multiple imputation will be performed to cope for missing data. A Cox proportional hazards model for time to pregnancy will be developed to estimate the cumulative chance of a live birth within 3 years after intake. To dynamically estimate the chance of an ongoing pregnancy, given the outcome of earlier pregnancies after intake, a logistic regression model will be developed.

ETHICS AND DISSEMINATION

The Medical Ethical Research Committee of the Leiden University Medical Centre approved this study protocol (N22.025). There are no risks or burden associated with this study. Participant written informed consent is required for both cohorts. Findings will be published in peer-reviewed journals and presentations at international conferences.

TRIAL REGISTRATION NUMBER

NCT05167812

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as the loss of two or more conceptions before 24 weeks of gestation (1). This condition affects approximately 1-3% of all fertile couples (2, 3). RPL is a highly heterogeneous condition with multiple known maternal risk factors, varying from auto-immune diseases (antiphospholipid syndrome (APS), antithyroid antibodies), parental balanced chromosomal translocations and congenital uterine abnormalities to advanced maternal age, maternal smoking and alcohol consumption. Besides these maternal factors, a potential contribution of paternal factors (such as male age, lifestyle factors and DNA fragmentation) has been recognized to add to the risk for miscarriages (4, 5, 6).

Despite extensive diagnostic work-up offered to couples with RPL, underlying risk factors can be identified in only 25-40% of couples (7, 8). Limited understanding of mechanisms underlying RPL has the consequence that effective treatment options are often lacking. When no evidence-based therapeutic options are available for couples with RPL, clinical management is primarily focused on providing supportive care. Supportive care and intensive pregnancy surveillance in the first weeks of gestation are assumed to be of influence in the prevention of new pregnancy loss (9).

Part of this supportive care is counselling on the prognosis and live birth rate of subsequent pregnancies in couples with RPL. Recently we conducted a systematic search to identify and assess the methodological quality of existing prediction models [Youssef et al, submitted for Fertility and Sterility 2021]. This review included the two most frequently used models which provide an estimate of subsequent chance of ongoing pregnancy/live birth in couples with unexplained RPL (10, 11). The model of Lund, et al. is actually not suitable for individual risk assessment, as stated by the authors themselves (11). The model of Brigham, et al. has been implemented in RPL care in the Netherlands and the United Kingdom, (10, 12, 13). These studies however did not follow the nowadays recommended TRIPOD guideline in the development and reporting of the model (14). For example, neither of the studies were internally nor externally validated and this could influence the validity and performance of the model. Recently, we showed that the

Brigham prediction model has poor performance in a Dutch RPL cohort, possibly due to a low number of patients included and a substantial change of the RPL population since 1999, in light of changes in defining unexplained RPL (15).

Most studies only concentrate on the first pregnancy after intake as primary outcome of the model, which lacks future perspective for couples with RPL. In addition, all earlier prediction models focused on the unexplained RPL population and on maternal predictors. None of them incorporated different causes for RPL, nor did they include paternal factors to establish a prediction specific to individual couples (16).

Individual couples with RPL now have an unclear prognosis of future success in terms of having a live birth. The aim of the current project is therefore to develop a prediction model that is able to provide tailormade estimations of pregnancy success in couples with both unexplained and explained RPL, and secondarily to develop a dynamic model that adjusts future chances based on pregnancies after intake.

STUDY OBJECTIVES

PRIMARY OBJECTIVE

To predict the chance of a live birth within three years after intake in couples with unexplained RPL.

SECONDARY OBJECTIVES

- To predict the chance of an ongoing pregnancy (>12 weeks) in the next pregnancy in couples with unexplained RPL.
- To predict the chance of a complicated pregnancy in couples with unexplained RPL (preeclampsia, HELLP, eclampsia, gestational diabetes, gestational hypertension, preterm birth, low birth weight).
- To predict the chance dynamically of a live birth given the outcome of a pregnancy after intake.
- To predict the chance of above outcomes in couples with a known cause for RPL.

METHODS AND ANALYSIS

STUDY DESIGN

A multicenter hospital-based prospective and retrospective cohort study to develop a prediction model. This study has a total expected duration of 5 years (Figure 1).

ELIGIBILITY CRITERIA

Couples with the following criteria at intake visit will be included:

1. RPL in the current relationship: defined as the loss of ≥ 2 preceding pregnancies. These pregnancy losses include:
    - All pregnancy losses before the 24th week of gestation verified by ultrasonography or uterine curettage and histology
    - Non-visualized pregnancies (including biochemical pregnancy losses and/or resolved and treated pregnancies of unknown location), verified by positive urine or serum human chorionic gonadotropin (hCG)
    - Both consecutive and non-consecutive pregnancy losses
2. Dutch or English speaking by either the male or the female of the couple
3. Couples with females aged ≤42 years

Couples will be excluded in case of mental or legal incapability of either male or female, or in case of < 2 pregnancies in current relationship.

Table 1 | Collection of clinical characteristics

Female	Date of birth, female age, alcohol consumption, smoking, caffeine intake, drugs intake, exercise pattern, education, BMI, blood pressure, general medical history (hypertension, diabetes mellitus, surgeries, earlier blood transfusions), use of medication, ethnicity and family history.
Male	Date of birth, male age, alcohol consumption, smoking, caffeine intake, drugs intake, exercise pattern, education, BMI, general medical history (hypertension, diabetes mellitus, surgeries etc.), use of medication, ethnicity and family history.
Obstetric history	Parity, number of miscarriages, ectopic pregnancies or induced abortions, mode of conception, mode of delivery of previous births, gestational age at previous births, birth weight of children of previous births.
RPL examination	Presence of APL (anticardiolipin IgG and IgM, β2 glycoprotein I antibodies IgG and IgM, and lupus anticoagulant), presence of thyroid antibodies, parental chromosomal abnormalities and presence of congenital uterine anomalies.

BMI: body mass index; RPL: recurrent pregnancy loss.

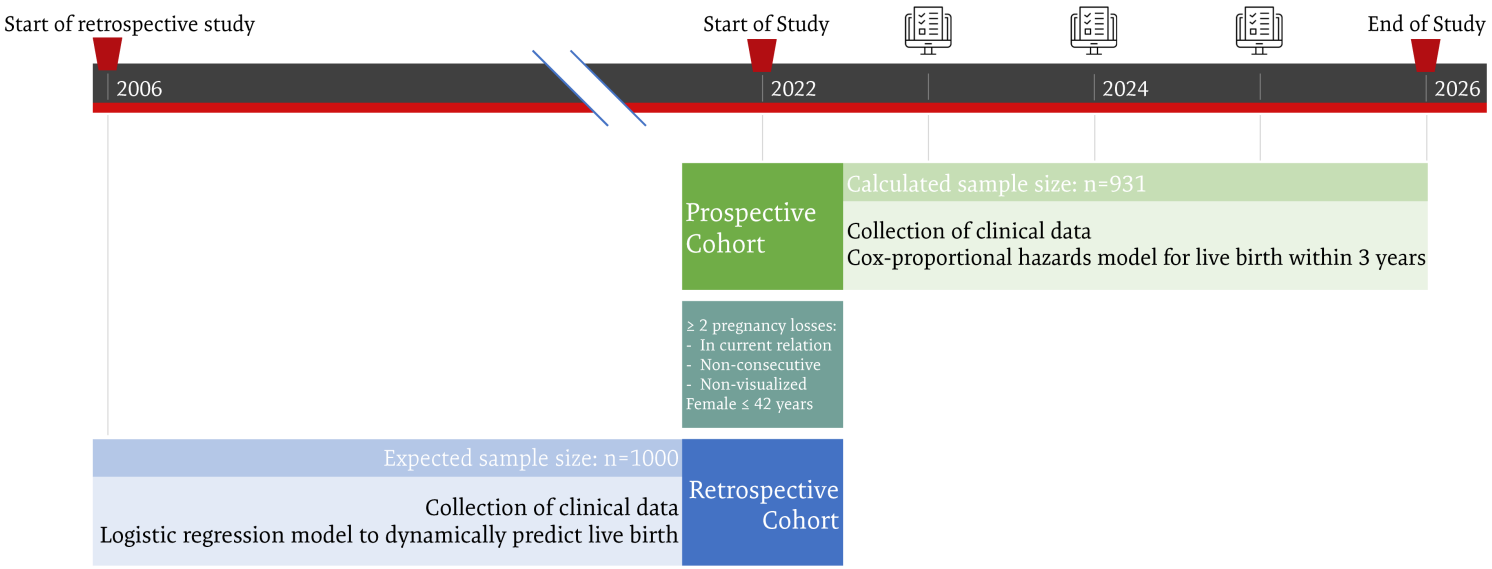

Figure 1: Schematic diagram of study design

STUDY POPULATION AND RECRUITMENT

RPL couples that visit the RPL outpatient clinic of the Leiden University Medical Centre (LUMC), or early pregnancy unit of the Erasmus University Medical Centre (Erasmus MC) or Amsterdam University Medical Centre (AUMC) will be assessed for eligibility. The LUMC is the coordinating centre. After referral, couples will have an intake at one of the aforementioned centres, where they will be invited to participate in this study. If eligibility criteria are met, and in case of consent, couples will be selected for inclusion. In addition to this prospective inclusion of patients, couples that have visited the aforementioned clinics between 2006 and 2021 will be included retrospectively.

Couples will receive written information about both the prospective and retrospective cohort, and a concomitant informed consent form. The informed consent consists of a request to obtain data from their medical records for this study, together with a request to obtain data from other medical professionals in case pregnancies were monitored in other centres. Study information underlines that participation is voluntary, and that couples are free to withdraw from the study at any time point without any consequences.

STUDY PROCEDURES

General medical history, lifestyle data and obstetric history will be collected for all couples (see table 1). Data will be collected during the initial intake visit. Uniformity in data collection between the participating centres will be ensured through templates. Digital surveys will be sent to participating couples to obtain additional data. All information will be stored in the electronic data capture software Castor EDC.

Couples participating in the prospective cohort will be followed for a total of 5 years after initial visit. Annual questionnaires will be digitally sent to obtain data of new pregnancies and/or changes in health or lifestyle. If follow up has taken place in one of the participating centres, couples will not have to fill in these questionnaires, but data will rather be obtained during consultation. Couples participating in the retrospective cohort will receive an online questionnaire in case of missing data.

CONTROL OF BIAS

According to the PROBAST-tool (17), risk of bias in prediction model development studies can be divided into four domains: participants, predictors, outcome and analysis. Study population is clearly defined, minimizing selection bias in the participants domain. As clinicians in the participating centres perform intakes in a semi-standardized manner, predictors will be assessed in a similar way for all participants. The outcome is clearly defined and determined: urine or serum hCG

measurement or heartbeat on ultrasound determine an ongoing pregnancy. To ensure that the analysis domain is not at risk of bias, the PROBAST-items of that domain will be followed. For the retrospective cohort, there is a risk of recall bias. Since intake visits are semi-structured, information at baseline is moderately similar across all inclusions. For additional information that has to be collected retrospectively, we aim to minimize recall bias by avoiding recall periods longer than five years.

SAMPLE SIZE CALCULATION

The method of Riley et al. for the sample size calculation in prediction models is used (18). This method consists of four steps and four different sample sizes, after which the largest one is selected as the study sample size. The four steps ensure a precise estimate of the overall outcome risk, predicted values with a small mean error across all individuals, a small required shrinkage of predictor effects and a small optimism in apparent model fit. Using an anticipated outcome proportion of 0.65 (live birth), 12 predictor parameters, a shrinkage of 0.9 and an anticipated R2cs of 0.1089, the largest sample size and thus this study's sample size is 931.

STUDY OUTCOMES

The following predictors were selected based on current literature, and will be assessed at intake (8, 10, 11, 19, 20, 21):

- Female age as a continuous variable
- Male age as a continuous variable
- Female BMI as a continuous variable
- Male BMI as a continuous variable
- Current female smoking as a categorical variable
- Current male smoking as a categorical variable
- Number of pregnancy losses as a categorical variable (2, 3, 4 and 5 or more)
- Heartbeat on ultrasound in obstetrical history as a binary variable
- ART in previous pregnancies as a binary variable
- Identification of an associated RPL factor as a binary variable

The following outcomes will be studied:

- Live birth within three years after initial intake visit (defined as the birth of a living child after 24 weeks gestation)
- Pregnancy outcomes since intake

- Time to pregnancy since intake
- Time between pregnancies since intake
- Pregnancy complications since intake

STATISTICAL ANALYSIS PLAN

For the primary outcome (live birth within three years after intake), we will develop a Cox proportional hazards model for time to pregnancy, including couples without full 3- or 5-year outcome information. For the secondary outcome, a logistic regression model for the binary outcome live birth in couples who conceived after their RPL intake will be developed. This will be used to dynamically predict live birth, given the outcome of pregnancies after intake

We will consider both simple linear and non-linear (restricted cubic splines) functions for continuous variables. The best fitting model is selected based on the Akaike Information Criterion which reflects the trade-off between information and model complexity (variable selection). Measurement of the AUC, the Brier score, the Brier skill score, and calibration of the model will be performed (Model performance). Internal validation will be performed using the bootstrapping method.

To cope with analysis of missing values (missing at random, missing completely at random), multiple imputation will be performed. Once the dataset is complete, cross validation of the previously selected variables will be performed, variables with a low predictive strength will be excluded.

External validation will be performed using data of Dutch academic hospitals which have not participated in this study.

PATIENT AND PUBLIC INVOLVEMENT

The Dutch association for patients with fertility problems (Freya) was consulted during the development of the study protocol. Study information will be published on their website, and information on progress and results will be presented to patients during meetings organized by Freya.

ETHICS AND DISSEMINATION

This study will be conducted according to the principles of the Declaration of Helsinki. The Medical Research Ethics Committee of the Leiden University Medical Centre provided ethical approval for this study (N22.025). There are no risks or burden involved in this study. All data will be collected during regular hospital visits or via questionnaires. Eligible couples will have sufficient time to decide on participating in this study, after having received written information. The Castor

EDC database of the OPAL study will contain all clinical and survey data. This database will not include directly traceable patient data. The findings of this study will be disseminated via peer reviewed publications and presentations at international conferences.

DISCUSSION

The perspective of a live birth is one of the most important aspects of RPL. Prognostic counselling plays a very important role in the RPL clinical practice, especially in the absence of an underlying risk factor and with the lack of treatment options. Different prognostic tools exist and are implicated in RPL care in the Netherlands and the United Kingdom, but these tools often are often of low quality [Youssef et al, submitted for Fertility and Sterility 2021].

In order to enable prediction of a live birth within three years or longer after initial intake visit, or to dynamically predict the chance of a live birth, a longer follow-up period is necessary. In this study proposal we will therefore include our patients not only prospectively, but also retrospectively. Retrospective inclusion is however known for recall bias. The initial intake visit is according to a semi-structured interview, thus minimizing differences between inclusion data across the retrospective cohort. In case of missing data, we will aim to minimize recall bias by avoiding recall periods longer than five years.

Another limitation of this study regards the predictors included in the model. There are various factors that are associated to RPL (such as sperm DNA fragmentation), that could possibly improve model performance, but we currently lack data to include these factors in a prediction model (22). Secondly, the predictor "identification of an associated RPL factor" does not specify the associated factor, something that would help counselling RPL couples. Of course, as there are several factors that could be categorized, the sample size needed for the inclusion of these factors would be much higher.

The ultimate goal of this study is therefore to accurately predict chances for future successful pregnancies, in order to aid expectation management, and provide a perspective for RPL couples. The outcomes of this study will provide tailormade and individual prognostic assessments of live birth in couples with RPL, and will have to be externally validated to ensure generalizability.

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CHAPTER 10

SUMMARY AND GENERAL DISCUSSION

Although RPL has been studied for almost a century, it is still a poorly understood condition that has a large impact on couples, which is intensified with each further loss experienced. Ideally, all underlying mechanisms of RPL are known and effective therapies targeted at these mechanisms are available. In the majority of RPL patients, the cause for RPL is aneuploidy, which lacks therapeutic options. In women with higher number of pregnancy losses, the proportion of aneuploid embryos could possibly be lower (1). It leaves the clinician to guide couples without having targeted therapeutic options, in which counselling towards future pregnancies is key. In this thesis we therefore aimed to identify current RPL practice to get a better understanding of the different concepts of definition, investigations and treatments in RPL, to appraise existing evidence that could impact counselling of RPL couples in order to individualize RPL care and management.

In summary, in this thesis we conclude that practice variation in clinical management of couples with RPL is present, both on local, national level as well as on international scale. Several barriers exist for RPL guideline implementation in the Netherlands; we identified possibilities to focus on implementation strategies. We found that currently existing prediction models, that estimate the chance of future live birth in women with RPL are not accurate, and should not be used in daily clinical practice. These results opened up various new research questions to be explored, one of which is the development of a new prediction model for which the study protocol is included in this thesis.

IDENTIFYING

Practice variation is present between various countries (2). This is particularly the case for medical conditions for which evidence regarding investigations and treatments are not based on undeniable evidence. On the other hand, little was known on practice variation that exists within a country, even in the presence of a national guideline. **Chapter 2** shows a comparison of seven local Dutch RPL protocols that have been compared to each other and to the Dutch NVOG guideline (3). Although the differences found between protocols were merely in the details of performing investigations, it reflects that practice variation is even present on a local, national level. It could leave couples with questions why different strategies

are available in different hospitals, which might lead to cross-border reproductive care (CBRC). Some practice variation is justified, for example because of varying patient characteristics such as ethnicity and accompanied risk profiles for certain diseases, and varying access to health care. Unwarranted practice variation however could impact various stakeholders, as effective care could potentially be underused and ineffective care could be overused. This could push healthcare costs up, while patient outcomes remain the same or even worse (4, 5). The emphasis should therefore not lie on clinicians' opinions, but rather on patient preferences after careful consideration of all medical evidence.

In **chapter 3** we analysed practice variation on an international level. We aimed to describe a methodology assessment (AGREE II) (6), summarize and compare the recommended definitions, risk factors, investigations and therapies of three prominent guidelines considering RPL. We found discrepancies between the guidelines across all aforementioned domains. Risk factors and investigations are generally similar between the compared guidelines, and the found discrepancies can be explained partly by their methods of development and the time of their publication, as well as the lack of strong evidence on some clinical aspects of RPL. This lack of evidence could also explain differences in treatment recommendation for uterine malformations and hereditary thrombophilia, for example. In general, the differences found in investigations and treatment recommendations create practice variation that could lead to CBRC. RPL couples are very much aware of investigations and treatments that are performed in other countries, and often seek these as a way of last resort, as they are in great distress of not being able to carry a pregnancy to term. Of course, psychological counselling, to support these couples in their distress and frustration, plays an essential role here. Additionally however is a universal, evidence-based RPL guideline derived from large associations such as the European Society for Human Reproduction and Embryology (ESHRE), Royal College of Obstetricians and Gynaecologists (RCOG) and American Society for Reproductive Medicine (ASRM) to ensure that couples with RPL all over the world receive comparable and evidence-based investigations and treatment options (7-10). In fact, only one guideline is needed, as all societies aim to develop an evidence-based guideline. A lot of work is being put into the development of these guidelines, which if bundled together might achieve

this universal guideline which will improve PRL practice variation across many countries. Of course, not all the recommendations are applicable to all populations worldwide, but if countries are similar in terms of medical services and populations, guidelines could be unified and tweaked according to local healthcare structures and organizations. One of the major hurdles in the universal application of one guideline is the inconsistency of the definition criteria of RPL, which appears to be a discussion based on opinions rather than evidence. If not internationally consistent, it will inherently lead to discrepancies in therapy of RPL. The definition will also have significant resource implications, as it will define when and when not to start performing investigations.

The previous chapters have shown that guidelines differ on both a national as well as international level. As the Dutch RPL guideline is currently being adapted from the ESHRE guideline, we aimed to detect possible barriers of implementing a new evidence-based guideline. **Chapter 4** describes a questionnaire study conducted across all gynaecology and obstetrics clinics in the Netherlands that identifies current clinicians' management and views on RPL practice. We observed that Dutch clinicians generally adhere to evidence-based investigations and therapeutic interventions in RPL care, but there is room for improvement. The main differences in guideline recommendation and clinician's RPL practice lies in the investigations performed and treatments considered, such as couple karyotyping, hereditary thrombophilia screening, thyroid function and auto-immunity testing and β2-glycoprotein antibodies testing in the context of APS. Differences were also found in APS treatment, where the starting order of LMWH and aspirin is based on expert opinion in the absence of evidence. The most frequently described non-evidence-based treatments were progesterone and aspirin in unexplained RPL. The use of aspirin or LMWH is not recommended in patients with unexplained RPL, as no significant benefits for any – or combination - of these anticoagulants was shown in comparison to placebo (11). The use of progesterone has been extensively studied for patients with unexplained RPL. The recommendation of the ESHRE guideline to not recommend progesterone treatment is based on the most recent and high-quality trial (12). In this chapter however, we were not able to examine the reason behind the differences in RPL practice in

this chapter. Future interview studies could be conducted to clarify why clinicians choose to offer non-evidence-based care.

As stated, and based on the present practice variation on local, national and international level, the development of one evidence-based RPL guideline is needed, that might be tweaked according to local healthcare structures and organizations. However, the existence of such an ideally available guideline is not enough, as dissemination of new guidelines is found to be insufficient to achieve proper guideline adherence. Implementation strategies are necessary to ensure guideline adherence (13, 14). The objective of **chapter 5** was to test a multi-faceted implementation strategy to improve healthcare professional's adherence to the RPL guideline. We found that four elements were directly related to higher adherence; the specified medical chart file, patient questionnaire, pocket card and electronic decision program. Adherence was significantly higher on most indicators on diagnostics and counselling. Prior to the strategy, 9 out of 21 measurable indicators showed an adherence below 50%, and after using implementation strategies, adherence was below 50% for just three out of 21 indicators. The highest increase was measured for selective screening for thrombophilia (+37%, Odd Ratio (OR) 5.2, 95% Confidence Interval (CI) 3.6-7.6). For counselling, the highest increase was measured for advising patient and partner to quit smoking (+50%, OR 13, 95% CI 4.8- 33). These strategies resulted in health care costs reduction of 206,916 euros annually in the four participating centres. This cost reduction, when extrapolated to all RPL clinics in the Netherlands, would potentially be even higher.

In the previous chapters we have identified several lessons in translating theoretical RPL guidelines to daily RPL practice. Both literature and clinical experience teaches that RPL is a frustrating condition with significant negative psychological impact on the patient. Many patients may feel the need to explore alternative care in other centres, both nationally and internationally. Organizing RPL care in a unified way may therefore also diminish this necessity felt by patients to turn to multiple, cross border opinions. This idea is supported by a comparable study to **chapter 4**, where Manning et al. showed equivalent results regarding practice variation (15). Their arguments revolve around the fact that in many practices no dedicated RPL specialists are present. A lead RPL clinician within each hospital or clinic could reduce variation and could result in a consistent approach of

managing RPL couples. Continuing onwards, differences exist between countries as well, which is probably the largest incentive for CBRC in RPL couples. Where options for investigations and treatments may be limited in one country, couples could travel to other countries in hope to increase their chances of a successful pregnancy. It is important for clinicians to realize that differences in RPL guidelines exist, increasing the number of investigations and treatments offered to couples attending RPL clinics. Knowledge of these differences should then be turned into counselling couples, assuring them that even though other clinics might provide other options, that current clinical practice is the best care possible. As mentioned earlier, a universal guideline should negate this issue of national healthcare societies recommending different strategies. Moreover, when obstetrics and gynaecology societies develop such a guideline together, this could ultimately be more cost-efficient, compared to these societies developing a guideline separately. In this way, societies could collectively present the same evidence-based guideline, adapted to the facilities of each country.

In chapter 5 we have also identified that implementation strategies for new guidelines are effective in increasing guideline adherence. Literature has shown that dissemination of guidelines alone is not enough for proper adherence, but no gold standards exist for developing strategies aiding implementation of updated or new guidelines. Lack of adherence to guidelines could result in under, as well as over diagnostics and treatments, which in turn leads to increasing health care costs. This is not only a theory, but the efficacy of implementation strategies is proven in chapter 5.

Overarching societies like the ESRHE could play a central role in implementation strategies. Next to the development of a unified evidence-based guidelines, every guideline should contain an implementation section. The efficacy of selected implementation strategies could be studied and adapted in the future for updated versions of the guideline. The ESHRE as overarching European society could work more closely with local societies to translate guidelines, and finetune implementation strategies according to the need of a specific obstetrics and gynaecology society. Working together will eventually lead to less practice variation, higher guideline adherence and therefore cost reduction and higher quality of care.

APPRAISING

Through the years, RPL definition and associated risk factors have been discussed extensively. There is no pathophysiological prove that distinguishes between women with two and women with three or more pregnancy losses, however there is some evidence that the probability of having certain associated factors such as APS and carrier status of structural chromosomal abnormalities are not different (16, 17). Chapter 6 provides insight in the occurrence of RPL associated risk factors, as well as a comparison in those with two pregnancy losses, and those with three or more pregnancy losses. We found that RPL associated factors occur with equal frequency in those two groups. Appraising this cohort provides evidence towards choosing two pregnancy losses as definition of RPL. Having established that the known risk factors are comparable across the two groups, one could argue that women with two pregnancy losses in their obstetric history should be eligible for RPL investigations and counselling, especially knowing that RPL couples carry a deep burden of not being able to successfully reproduce. By presenting at an early stage in their reproductive path with two failed pregnancies in hindsight, supportive care could be initiated at an early time point.

One of the most asked questions of RPL couples relates to their future: "will we carry a pregnancy successfully resulting in a live birth? And if so, how high is our chance of doing so?"

In chapter 6 we described that chances of having a future ongoing pregnancy are relatively high even though this chance decreases with an increasing number of pregnancy losses. In 83.5% of women included in the study, an ongoing pregnancy was reported). It is important to note that an ongoing pregnancy was defined as a pregnancy continuing after the 12th week of gestation. This high chance of a future ongoing pregnancy could be a comforting thought for couples suffering from RPL, however it does not indicate the chance of having a live birth, which is the ultimate goal for RPL couples. Furthermore, this prediction is for the whole cohort, and could not be applied on individual couples. To be able to finetune this, a prediction model is needed. Prediction models combine characteristics of individual patients to provide information about the likelihood of uncertain outcomes. Predicting live birth rate of subsequent pregnancies in RPL is an essential

part of supportive care, as information manages the expectations of the couple and improves their ability to make an informed decision regarding further pregnancy attempts.

Before using prediction models in clinical practice, it is of utmost importance that these models have been developed accurately, and have been validated internally and externally. Several prediction models for RPL exist and used in clinical practice, however they have never been critically appraised. Therefore, in **chapter 7** a systematic review has been conducted, aiming to find existing RPL prediction models and critically appraise them. As knowledge on development and validation of prediction models has increased throughout the years, tools have been published aiming to provide guidance for reporting all necessary prediction study items and for critically appraising risk of bias in prediction studies (18, 19). We showed that the seven included prediction models did not follow the recommended steps for prediction model development, including internal validation for the prevention of overfitting. None of the studies performed a sample size calculation, and retrospective sample size calculation showed that three studies were too small for the included number of prediction parameters (20-26). It is especially noteworthy that the preferred prediction models by the ESHRE for clinical use, Brigham et al. and Lund et al. (21, 24), were both shown to be at high risk of bias, reasons thereof including insufficient sample size and categorization of predictors. Other limitations include not reporting missing data, which in case of systematic reasons could lead to selection bias, and the inclusion of patients that have been treated with various therapeutics, which could impact the prognostic effect of selected predictors.

Besides limitations based on reporting of data, there are also limitations concerning the statistical validity and applicability of prediction models. Usually, predictive performance of prediction models is described using discrimination and calibration. Only two studies reported a c-statistic, a measure of the prediction model's discriminative ability, namely 0.642 for subsequent pregnancy risk calculation in Sugiura-Ogasawara's study and 0.62 in Bashiri's study, both considered moderately discriminative (20, 26). None of the included study in this chapter performed calibration of their model, which is arguably more important than assessing its discriminative ability as calibration assesses the fitness of data to the developed model.

Before using clinical prediction models in clinical practice both internal as external validation should be performed. Internal validation is important as the model is expected to perform well on its own data, since, the model was designed to fit the development data. Without internally validating a prediction model, overfitting could exist, meaning that the model will be less accurate when tested in a new RPL population. External validation is similar to internal validation, but performed in an unrelated cohort. It is needed to confirm that the developed prediction model is able to predict the outcome well enough in unrelated individuals to those of the development cohort. If external validation is performed, and the prediction model was found to perform well, implementation of the model could be considered.

In short, based on the results of this systematic review, at this moment we cannot recommend any prediction model in clinical practice. Though the use of prediction models is recommended by various guidelines, it is important to perform external validation to make sure that couples are counselled correctly.

Based on the conclusions and advices we formulated in **chapter 7**; an external validation study was performed on the most widely used and recommended RPL prediction models. In **chapter 8** we aimed to validate the prediction models of Brigham et al. and Lund et al. We learned from **chapter 7** that the prediction model of Lund et al. was not intended for individual risk assessment and did not calculate the chance of future pregnancy success individually. We therefore decided to only externally validate the prediction model as described by Brigham et al. In a cohort consisting of 739 women, with similar characteristics compared to Brigham's cohort (mean age of 33.1 years and a median of three pregnancy losses at intake), we showed that the mean predicted pregnancy success rate was 9.8 percentage points higher in the Brigham model than the observed pregnancy success rate in the dataset (73.9% vs 64.0% (CI 95% for the 9.8% difference 6.3% – 13.3%)) (21). Performance was measured using calibration and discrimination, with calibration showing overestimation of the model and too extreme predictions (negative calibration intercept of -0.46 (CI 95% -0.62 – -0.31) and calibration slope of 0.42 (CI 95% 0.11 – 0.73)). This calibration slope of < 1 suggests that the estimated risks are too extreme, which translates to a stronger effect of the

predictors used by Brigham compared to the validation dataset. The c-statistic, describing the discriminative ability of the model was 0.55 (CI 95% 0.51 – 0.59). This value ranges from 0.5 to 1.0, with 0.5 indicating prediction based on pure chance and 1.0 indicating perfect prediction. In short; the model overestimates, has too extreme predictions and has a poor discriminative ability.

The results of the external validation of the prediction model as developed by Brigham et al. could be regarded as a consequence of the high risk of bias discovered in **chapter 7**. The accuracy of prediction models is often lower in an unrelated cohort (27) and this also relates to for Brigham's model. A small cohort of RPL patients, the lack of internal and external validation probably resulted in the poor performance of this model. Even after model updating using recalibration, which re-estimates all used coefficients, no improvement was possible. The results of this study suggest that the currently most widely used prediction model for couples with RPL leads to a model that cannot discriminate between patients with or without a successful future pregnancy.

The current model's base prognosis on only two predictors: the number of previous pregnancy losses and maternal age. This leads to the following question whether the predictive ability of the model will improve when taking additional candidate predictors into account. Actually, which predictors should be included in future RPL prediction models, and how well the RPL prediction model should be able to discriminate between those with and without the desired outcome? Regarding the first question, the answer is that predictor finding studies in RPL are scarce, and that selecting predictors for inclusion in RPL prediction models is often done on theoretical grounds, rather than on grounds of a scientifically proven predictive association. Of course, in the absence of such predictor finding studies, this is the next best solution for now.

Next, the question is how good the predictive ability of the model should be to be used in clinical practice. The second question could be answered in two ways, either based on the values a c-statistic can hold or based on the multifactorial process that leads to pregnancy, and eventually the success or failure of pregnancy. Ideally, the higher the c-statistic, the better. However, prediction studies in pregnancy often find c-statistics lower than

0.65. It is known that pregnancy is indeed a multifactorial process, that is still not fully understood in terms of what leads to pregnancy success and what leads to failure. One can therefore discuss whether a prediction model could ever have a discriminative ability much higher than those described in current day literature.

Besides these performance measures, differences in cohort characteristics are important to take in mind, including differences in definitions and cohort populations. This will remain present as prediction models are time-dependent owing to scientific advances and population changes. It is therefore important that future prediction studies take this into account and allow for updating.

INDIVIDUALIZE

Having identified clinical RPL practice and having appraised prediction models aimed at improving counselling, it is time to take a look at the future and pave a pathway for individualization of RPL counselling. **Chapter 9** combines all lessons learned from both clinical practice and prediction studies, presenting a protocol for the development and validation of a new RPL prediction model, aiming to precisely and accurately predict future chances of live birth in couples with RPL, in the group with and without underlying risk factors.

The primary objective of this model is to predict the chance of a live birth within three years after first consultation in couples with unexplained RPL. This outcome was defined as more clinically relevant for patients than just knowing the outcome of the first pregnancy after intake. Secondly, the aim of the model is to dynamically predict the chance of a live birth given any outcome of pregnancy after intake.

In our protocol we carefully considered development and reporting according to the TRIPOD statement in order to ensure a scientifically valid model (18). In addition, the PROBAST-tool will be used to ensure risk of bias across the various domains of the study is minimized as much as possible (28). By involving all stakeholders including clinicians and patient organizations for couples with fertility problems, we aim to create a supporting base for the use of this model in the future. A well designed and

easy to use tool caters to the likelihood of this model being implemented in daily RPL care.

For model development, we selected variables found in the systematic literature search described in **chapter 7**. We are however limited regarding the inclusion of predictors due to sample size requirements. Various factors associated to RPL could possibly improve model performance (such as sperm DNA fragmentation), but data backing these factors are currently lacking (29). As mentioned previously, it is important to keep scientific and population changes throughout time in mind, and update this model when needed.

The ultimate goal of this study is to accurately predict chances for future successful pregnancies, by using as much predictive information as possible from both male and female partner, in order to aid expectation management, and provide a perspective for RPL couples. The outcomes of this study will provide tailormade and individual prognostic assessments of live birth in couples with RPL. Over time, this model should be a living, dynamically changing tool that is updated through time according to the latest evidence identified, and should be continuously appraised to keep providing the best possible individualized counselling.

SUPPORTIVE CARE

Supportive care is central to the management of RPL, especially in those couples without an identifiable factor (30-32). Specialised RPL unites could arrange supportive care perfectly, consisting of both psychological and medical help. Part of medical supportive care consists of counselling on the prognosis and live birth rate in future pregnancies. This is important information for couples, as it could help manage expectations and aid couples into making informed decisions regarding future pregnancy attempts. Based on the findings in this thesis, after having appraised various prediction models, a few items follow to consider in RPL prediction studies.

First of all, the predictors considered should be extensively reviewed. Up till now, mainly age and previous pregnancy losses are included. Although predictor findings studies are lacking, there are several known factors influencing spontaneous pregnancy loss, which are often lifestyle related (smoking, high BMI (33, 34)). As half of the product of conception is derived

from the male partner, male predictor parameters should be considered as well (29).

Besides predictors, it is also important to reconsider the outcome that is being predicted. Clinical experience tells that couples are not just interested in the first pregnancy after intake, but want to know their perspective in a time range of 3 to 5 years. Live birth in 3-5 years would therefore be more relevant as outcome for RPL prediction models. This could also help the clinician in adjusting supportive care strategies according to the predicted chances and couples' preferences for supportive care (35).

The findings of chapters 6, 7 and 8 pave a path towards individualized RPL care, in which counselling should be adapted to the individual needs of each couple. This concept of individualized RPL care could shift current practices to a more value-based organization of RPL care, in which matters that patients value the most are more closely incorporated in RPL practice. Value-based healthcare is a healthcare system in which (multidisciplinary) care is organized around a patient's medical condition. It targets the outcomes that make the biggest difference to patients, while driving cost efficiencies within health services. RPL care could benefit from this system as patient outcomes and values are central to the provided care. By analysing current practices and interviewing RPL couples, a set of high priority values could be selected, to which RPL care could be adapted. By continuously evaluating patient outcomes, this system allows for improvements over time in this RPL care path.

CONCLUSIONS AND A LOOK TOWARDS THE FUTURE OF RPL CARE

This thesis has shed light on RPL practice and the management of RPL couples in need of counselling towards future pregnancies. Both clinical practice research and prediction research indicate that there is room for improvements in RPL practice and RPL counselling. We studied quality of care by diving into clinical practice variation and quality of counselling by diving into prediction research.

It could be defeating to know that a large proportion of couples with RPL seem to not have any identifiable risk factor. It is understandable that both clinicians and patients seek options outside of guideline recommendation, being desperate in grasping every bit of hope that might lead to a live birth. Acknowledging practice variation and reasons hereto might shift the focus of research to study subjects that will improve effective, evidence-based care and above all maintain one of the most important principles in medicine: first, do no harm. This could be applied to investigations and treatments performed in RPL couples, but is also on RPL research in which low quality research harms the way our medical society perceives new evidence. Research output should focus on quality instead of quantity, and be focused on those questions that are most urgently waiting to be discovered.

In the absence of effective treatment options that increase live birth rates in RPL couples, counselling towards future pregnancies plays a key role and enables couples to make an informed decision regarding further pregnancy attempts. This key role for prediction models will still be present when future treatment options are investigated or discovered, as these models could then evaluate the effects of these treatments on performance of the model. It is therefore of utmost importance that prediction models are well-developed and validated for use in clinical practice.

In an era of technological advancement at high rates, bringing societies, researchers and clinicians from all over the world more closely together than ever, it is time to step up and work together, to unify RPL care and to create collaborations that hugely impact RPL research which can lead to high impact publications that can unravel the mysteries of RPL.

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NEDERLANDSE SAMENVATTING & APPENDICES

NEDERLANDSE SAMENVATTING

Het krijgen van een miskraam is een van de meest voorkomende zwangerschapscomplicaties. Herhaalde miskramen worden doorgaans gedefinieerd als het spontane verlies van twee of meer zwangerschappen voordat de termijn van levensvatbaarheid wordt bereikt (24 weken). Zowel maternale als paternale aandoeningen en risicofactoren zijn geassocieerd met herhaalde miskramen, waaronder het antifosfolipidensyndroom, uterusanomalieën, abnormale karyotypering, sperma-DNA-fragmentatie en leefstijlfactoren. Het exacte aantal koppels met herhaalde miskramen is onbekend.

Ondanks het feit dat herhaalde miskramen al sinds de jaren '30 worden onderzocht, blijven er nog veel vragen onbeantwoord. Het onderliggende mechanisme van herhaalde miskramen is nog steeds niet ontrafeld. Onderzoek gericht op foetale chromosomen van miskraamweefsel toont aan dat in meer dan de helft van de miskramen sprake is van aneuploïdie (een verkeerd aantal chromosomen). Dit lijkt geassocieerd te zijn met oudere leeftijd, roken en obesitas, hoewel koppels met een hoog aantal miskramen juist vaker euploïde miskramen krijgen (foetus met het juiste aantal chromosomen). Ondanks uitgebreid onderzoek wordt er slechts in 25% van de koppels met herhaalde miskramen een oorzaak gevonden. Hierdoor blijft het voor medici moeilijk om te verklaren waarom sommige zwangerschappen succesvol verlopen en andere tot een miskraam leiden.

Er is veel discussie rondom het definiëren, onderzoeken en behandelen van herhaalde miskramen, hoewel er verschillende richtlijnen bestaan, waaronder die van de European Society for Human Reproduction and Embryology (ESHRE). Dit geldt des te meer voor koppels met onverklaarde herhaalde miskramen, waarbij geen onderliggende aandoening lijkt te zijn en (experimentele) behandelingen niet worden aanbevolen. Deze onenigheid leidt vaak tot onderzoeken en behandelingen die niet wetenschappelijk onderbouwd zijn, en tot verschillen in de aanpak van herhaalde miskramen tussen verschillende centra.

Deze praktijkvariatie komt tot uiting bij koppels die buiten Nederland zorg zoeken, waar ze uitgebreidere diagnostiek en mogelijke behandelingen kunnen krijgen, hoewel deze vaak wetenschappelijk niet bewezen effectief zijn. Behandelingen die niet bewezen effectief zijn, gaan daarom in tegen

het principe van "niet schaden". Het is logisch dat de psychologische belasting voor stellen met herhaalde miskramen toeneemt na elk zwangerschapsverlies. Het is daarom belangrijk om voldoende zorg te bieden.

Eén van de vormen van ondersteunende zorg bestaat uit het bieden van een toekomstperspectief betreffende de kans op een succesvolle zwangerschap. Deze informatie kan door stellen worden gebruikt bij het nemen van beslissingen over eventuele toekomstige zwangerschapspogingen. In de dagelijkse praktijk van zorg bij herhaalde miskramen bestaan er twee predictiemodellen die worden gebruikt om de kans op toekomstige zwangerschappen te voorspellen, namelijk het model van Lund et al. en het model van Brigham et al. Deze modellen worden genoemd in verschillende internationale richtlijnen. In deze modellen wordt de kans op een succesvolle zwangerschap geschat op basis van leeftijd en aantal eerdere miskramen. Hoewel deze modellen als van hoge methodologische kwaliteit worden beschouwd, hebben ze niet de aanbevolen richtlijnen gevolgd voor het ontwikkelen van een predictiemodel.

Predictiemodellen bieden clinici handvatten om informatie te verstrekken over het optreden van bepaalde uitkomsten. Vier elementen maken predictiemodellen effectief in de dagelijkse praktijk: ontwikkeling, validatie, evaluatie van de impact en updates.

Gewoonlijk zijn predictiemodellen afgeleid van een multivariabel regressiemodel voor dichotome uitkomsten. In de ontwikkelingsfase is het belangrijk dat de database waarop het model is gebaseerd groot genoeg is om betrouwbaarheid te garanderen. In het verleden werd de vuistregel "10 gebeurtenissen per prognostische factor" gebruikt. Er is veel discussie geweest in de wetenschappelijke literatuur over deze regel, waarbij de hypothese is dat het aantal gebeurtenissen per prognostische factor contextspecifiek is en afhankelijk is van meerdere factoren. Daarom heeft Riley et al. een vierstappenprocedure ontwikkeld om een voldoende grote steekproefomvang te berekenen en een model met weinig foutgevoeligheid te waarborgen.

Validatie van een predictiemodel is belangrijk om de generaliseerbaarheid van het model te testen. Hierbij wordt het ontwikkelde model gebruikt in een nieuwe externe dataset, waarbij de voorspellende prestaties van het

model worden onderzocht. Vervolgens kan het model geïmplementeerd worden en kan worden onderzocht hoe het model de patiëntuitkomsten en kosteneffectiviteit beïnvloedt. Tot slot kan het model worden bijgewerkt op basis van nieuwe inzichten.

In een poging om de kwaliteit van zorg voor stellen met herhaalde miskramen te verbeteren, richt dit proefschrift zich op twee hoofdgebieden. In deel I staat de aanwezigheid van verschillende richtlijnen en hun gebruik in de klinische praktijk centraal bij het begrijpen van de zorg voor herhaalde miskramen zoals die vandaag de dag wordt verleend. Het is belangrijk om (inter)nationale verschillen in de zorg voor herhaalde miskramen te bestuderen, omdat praktijkvariatie kan leiden tot een toenemend aantal patiënten dat zorg zoekt bij verschillende (inter)nationale centra, om uitgebreidere onderzoeken en verschillende niet-wetenschappelijk bewezen behandelingen te ondergaan. Deel II van dit proefschrift richt zich op het gebruik van prognostische modellen ter ondersteuning van stellen met herhaalde miskramen. Aangezien begeleiding bij toekomstige zwangerschappen een sleutelrol speelt in de zorg voor herhaalde miskramen, is het belangrijk dat clinici accurate informatie kunnen verstrekken. Na zorgvuldige evaluatie van beschikbare prognostische modellen zal een nieuw prognostisch instrument worden geïntroduceerd.

DIT PROEFSCHRIFT

Hoofdstuk 2 toont een vergelijking van zeven lokale Nederlandse protocollen voor herhaalde miskramen, die zijn vergeleken met de Nederlandse NVOG-richtlijn. Hoewel de gevonden verschillen tussen de protocollen voornamelijk betrekking hadden op de details van het uitvoeren van onderzoeken, weerspiegelt dit het feit dat praktijkvariatie zelfs op lokaal en nationaal niveau aanwezig is.

In **hoofdstuk 3** hebben we praktijkvariatie op internationaal niveau geanalyseerd. Ons doel was om de aanbevolen definities, risicofactoren, onderzoeken en therapieën van drie prominente richtlijnen voor herhaalde miskramen samen te vatten en te vergelijken. We hebben discrepanties gevonden tussen de richtlijnen op al deze genoemde gebieden. Risicofactoren en onderzoeken zijn over het algemeen vergelijkbaar tussen de vergeleken richtlijnen, en de gevonden verschillen kunnen deels worden verklaard door hun ontwikkelingsmethoden en het tijdstip van publicatie,

evenals het gebrek aan sterk bewijs voor sommige klinische aspecten van herhaalde miskramen.

Stellen met herhaalde miskramen zijn zich zeer bewust van onderzoeken en behandelingen die in andere landen worden uitgevoerd, en ze zoeken deze vaak als laatste redmiddel, omdat ze geen succesvolle zwangerschap kunnen bereiken. Er is behoefte aan een universele, op bewijs gebaseerde richtlijn voor herhaalde miskramen om ervoor te zorgen dat stellen met herhaalde miskramen over de hele wereld vergelijkbare en op bewijs gebaseerde onderzoeken en behandelingsopties krijgen. Natuurlijk zijn niet alle aanbevelingen van toepassing op alle bevolkingsgroepen wereldwijd, maar als landen vergelijkbaar zijn op het gebied van medische diensten en bevolkingssamenstelling, kunnen richtlijnen worden samengevoegd en aangepast aan lokale gezondheidszorgstructuren en -organisaties. Een van de belangrijkste hindernissen bij de universele toepassing van één richtlijn is de inconsistentie van de definitiecriteria voor herhaalde miskramen, die lijkt te worden bepaald door meningen in plaats van bewijs. Als dit niet internationaal consistent is, zal dit inherent leiden tot verschillen in de behandeling van herhaalde miskramen. De definitie heeft ook consequenties voor de beschikbare middelen, omdat dit bepaalt wanneer onderzoeken worden ingezet.

De voorgaande hoofdstukken hebben aangetoond dat richtlijnen verschillen, zowel op nationaal als internationaal niveau. Aangezien de ESHRE-richtlijn wordt aangepast voor gebruik in Nederland, hebben we geprobeerd mogelijke belemmeringen bij de implementatie van een nieuwe op bewijs gebaseerde richtlijn te achterhalen. **Hoofdstuk 4** beschrijft een vragenlijstonderzoek dat is uitgevoerd in alle gynaecologiepraktijken in Nederland, waarbij de huidige zorg voor herhaalde miskramen en de visie van clinici daarop zijn geïdentificeerd. We hebben waargenomen dat Nederlandse clinici over het algemeen de op bewijs gebaseerde onderzoeken en therapeutische interventies in de zorg voor herhaalde miskramen naleven, maar dat er ook ruimte is voor verbetering. De belangrijkste verschillen in richtlijnaanbeveling en de praktijk van clinici op het gebied van herhaalde miskramen liggen in de uitgevoerde onderzoeken en behandelingen, zoals karyotypering, screening op erfelijke trombofilie, schildklierfunctie en auto-immuniteit, en β2-glycoproteïne-antilichaamtesten in de context van het antifosfolipidensyndroom. In dit

hoofdstuk waren we echter niet in staat om de reden achter de verschillen in de zorg voor herhaalde miskramen te onderzoeken. Toekomstige interviewstudies zouden kunnen worden uitgevoerd om te verduidelijken waarom clinici ervoor kiezen om niet op bewijs gebaseerde zorg te bieden.

Hoofdstuk 5 had als doel een implementatiestrategie te testen om de naleving van de richtlijn voor herhaalde miskramen door zorgprofessionals te verbeteren. We hebben vastgesteld dat vier elementen direct verband hielden met een betere naleving: een gespecificeerd medisch dossier, een vragenlijst voor patiënten, een zakkaart en een elektronisch beslissingsprogramma. De naleving was aanzienlijk hoger voor de meeste indicatoren met betrekking tot diagnostiek en counseling. Deze strategieën resulteerden in een kostenverlaging van 206.916 euro per jaar in de vier deelnemende centra. Deze kostenverlaging zou mogelijk nog hoger zijn wanneer dit wordt geëxtrapoleerd naar alle centra die herhaalde miskramen zorg bieden. Uit de literatuur is gebleken dat het verspreiden van richtlijnen alleen onvoldoende is voor een goede naleving. Er bestaan echter geen gouden standaarden voor het ontwikkelen van strategieën ter ondersteuning van de implementatie van bijgewerkte of nieuwe richtlijnen. Overkoepelende organisaties zoals ESHRE kunnen een centrale rol spelen in implementatiestrategieën, waarin naast de ontwikkeling van richtlijnen ook een implementatiesectie kan worden opgenomen voor elke richtlijn. De effectiviteit van geselecteerde implementatiestrategieën kan vervolgens in de toekomst worden bestudeerd en aangepast.

In de loop der jaren is de definitie van herhaalde miskramen en de bijbehorende risicofactoren uitgebreid besproken. Er is geen pathofysiologisch bewijs dat onderscheid maakt tussen vrouwen met twee zwangerschapsverliezen en vrouwen met drie of meer miskramen, maar er is enig bewijs dat de kans op bepaalde geassocieerde factoren, zoals het antifosfolipidensyndroom en het drager zijn van structurele chromosomale afwijkingen, niet verschillend is. Hoofdstuk 6 biedt inzicht in deze oorzaken en geassocieerde factoren. We hebben vrouwen met twee miskramen en vrouwen met drie of meer miskramen vergeleken, en in ons cohort hebben we vastgesteld dat de risicofactoren voor herhaalde miskramen met dezelfde frequentie voorkomen in beide groepen. Deze bevinding biedt een basis om twee miskramen als definitie voor herhaalde miskramen te hanteren. Aangezien de frequentie van risicofactoren

vergelijkbaar is tussen de twee groepen, kan worden overwogen om onderzoek naar herhaalde miskramen te starten vanaf twee miskramen, vooral omdat bekend is dat stellen met herhaalde miskramen psychologische last ervaren vanwege het niet kunnen voortplanten. Op deze manier kan tijdig ondersteunende zorg worden geboden.

Een van de meest gestelde vragen van stellen met herhaalde miskramen heeft betrekking op hun toekomst: "Zullen we een zwangerschap succesvol kunnen dragen die resulteert in de geboorte van een levend kind? En zo ja, hoe groot is onze kans hierop?" Deze vragen kunnen worden beantwoord met behulp van predictiemodellen. Voordat deze modellen in de praktijk kunnen worden gebruikt, is het van belang dat ze nauwkeurig worden ontwikkeld en zowel intern als extern worden gevalideerd. Er bestaan verschillende voorspellende modellen voor herhaalde miskramen die in de klinische praktijk worden gebruikt, maar ze zijn nog nooit kritisch beoordeeld. Daarom is in **hoofdstuk 7** een systematische review uitgevoerd om bestaande predictiemodellen voor herhaalde miskramen te vinden en kritisch te beoordelen. Naarmate de kennis over de ontwikkeling en validatie van predictiemodellen in de loop der jaren is toegenomen, zijn er tools gepubliceerd die als leidraad dienen voor het rapporteren van alle benodigde items en voor het kritisch beoordelen van het risico op vertekening (bias) in studies die predictiemodellen proberen te ontwikkelen. We hebben aangetoond dat de zeven geïncludeerde predictiemodellen de aanbevolen ontwikkelingsstappen niet hebben gevolgd, waaronder interne validatie. Geen van de studies heeft een berekening van de steekproefgrootte uitgevoerd. Een retrospectieve berekening van de steekproefgrootte toonde aan dat drie studies te klein waren voor het aantal opgenomen voorspellende parameters. Het is met name opmerkelijk dat de predictiemodellen die door de ESHRE worden aanbevolen voor klinisch gebruik, namelijk die van Brigham et al. en Lund et al., beide een hoog risico op vertekening hadden, onder andere vanwege onvoldoende steekproefgrootte en categorisatie van voorspellers. Andere beperkingen zijn het niet rapporteren van ontbrekende gegevens, wat kan leiden tot selectiebias, en het opnemen van patiënten die zijn behandeld met verschillende therapeutische opties, wat van invloed kan zijn op het voorspellende effect van geselecteerde voorspellers.

Doorgaans worden de voorspellende prestaties van predictiemodellen beschreven aan de hand van discriminatie en kalibratie. Slechts twee studies rapporteerden de c-statistiek, een maat voor het onderscheidend vermogen van het voorspellende model. Geen van de geïncludeerde studies in dit hoofdstuk voerde kalibratie van hun model uit, wat mogelijk belangrijker is dan het beoordelen van het onderscheidend vermogen, omdat kalibratie de mate van overeenstemming van de gegevens met het ontwikkelde model beoordeelt.

Voordat klinische voorspellingsmodellen in de praktijk worden gebruikt, moet zowel interne als externe validatie worden uitgevoerd. Interne validatie is belangrijk omdat van het model wordt verwacht dat het goed presteert op zijn eigen gegevens, aangezien het model is ontworpen om bij de ontwikkelingsgegevens te passen. Zonder interne validatie van een voorspellend model kan er overfitting optreden, wat betekent dat het model minder nauwkeurig zal zijn bij testen in een nieuwe populatie met herhaalde miskramen. Externe validatie lijkt op interne validatie, maar wordt uitgevoerd in een niet-gerelateerd cohort. Het is nodig om te bevestigen dat het ontwikkelde predictiemodel de uitkomst voldoende goed kan voorspellen bij niet-gerelateerde patiënten in vergelijking met het cohort waarop het model is ontwikkeld. Als externe validatie wordt uitgevoerd en het voorspellingsmodel blijkt goed te presteren, kan overwogen worden om het model te implementeren.

Op basis van de conclusies en adviezen die we hebben geformuleerd in hoofdstuk 7, is er een externe validatiestudie uitgevoerd op de meest gebruikte en aanbevolen voorspellingsmodellen voor herhaalde miskramen. In **hoofdstuk 8** hebben we geprobeerd de voorspellingsmodellen van Brigham et al. te vergelijken met een cohort van 739 vrouwen met vergelijkbare kenmerken als het cohort van Brigham (gemiddelde leeftijd van 33,1 jaar en een mediane van drie zwangerschapsverliezen bij intake). We hebben aangetoond dat het voorspelde gemiddelde zwangerschapssuccespercentage in het Brigham-model 9,8 procentpunten hoger lag dan het waargenomen zwangerschapssuccespercentage in de dataset (73,9% vs. 64,0%). De prestaties werden gemeten aan de hand van kalibratie en discriminatie, waarbij de kalibratie liet zien dat het model overschatting vertoonde en te extreme voorspellingen deed. Tevens zijn de geschatte risico's te extreem.

Het onderscheidend vermogen van het model bedroeg 0,55. Deze waarde kan getallen aannemen tussen 0,5 en 1,0, waarbij 0,5 duidt op voorspellingen op basis van puur toeval en 1,0 duidt op perfecte voorspelling. Kortom, het model overschat, maakt te extreme voorspellingen en heeft een slecht onderscheidend vermogen.

De resultaten van de externe validatie van het voorspellingsmodel ontwikkeld door Brigham et al. kunnen worden beschouwd als een gevolg van het hoge risico op bias dat in **hoofdstuk 7** is ontdekt. De nauwkeurigheid van voorspellingsmodellen is vaak lager in een niet-verwant cohort, en dit geldt ook voor het model van Brigham. Een klein cohort van herhaalde miskramen patiënten en het ontbreken van interne en externe validatie hebben waarschijnlijk geleid tot de slechte prestaties van dit model. Zelfs na modelaanpassing door middel van herkalibratie, waarbij alle gebruikte coëfficiënten opnieuw worden geschat, was geen verbetering mogelijk. De resultaten van deze studie suggereren dat het momenteel meest gebruikte voorspellingsmodel voor stellen met herhaalde miskramen niet in staat is om onderscheid te maken tussen patiënten met wel of zonder een succesvolle toekomstige zwangerschap.

De huidige modellen baseren hun prognose alleen op twee voorspellers: het aantal eerdere miskramen en de leeftijd van de moeder. Dit leidt tot de vraag of de voorspellende capaciteit van het model zal verbeteren wanneer rekening wordt gehouden met aanvullende potentiële voorspellers. Er zijn echter weinig predictor studies uitgevoerd in herhaalde miskramen, en het selecteren van predictoren voor opname in predictiemodellen gebeurt vaak op theoretische gronden. Dit is momenteel de beste aanpak in afwezigheid van dergelijke predictor studies.

De vraag hoe goed een herhaalde miskramen voorspellingsmodel moet zijn om onderscheid te maken tussen degenen met en zonder het gewenste resultaat kan op verschillende manieren worden beantwoord. Een benadering is gebaseerd op de mogelijke waarden die een c-statistiek kan hebben, waarbij geldt dat een hogere c-statistiek beter is. Echter, predictor studies in de zwangerschap hebben vaak c-statistieken lager dan 0.65. Het is bekend dat zwangerschap een multifactorieel proces is dat nog niet volledig wordt begrepen in termen van wat leidt tot zwangerschapssucces en wat leidt tot het doormaken van een miskraam. Daarom is het

twijfelachtig of een predictiemodel ooit een hoger onderscheidend vermogen zal hebben dan wat momenteel in de literatuur wordt gevonden.

Hoofdstuk 9 presenteert een protocol voor de ontwikkeling en validatie van een nieuw predictiemodel voor herhaalde miskramen, met als doel de kans op een succesvolle zwangerschap in de toekomst bij stellen met herhaalde miskramen nauwkeurig te voorspellen. Het model richt zich zowel op stellen met als zonder onderliggende risicofactoren. Het primaire doel van het model is om de kans op een succesvolle geboorte binnen drie jaar na de eerste consultatie te voorspellen, wat klinisch relevanter is voor patiënten dan alleen de uitkomst van de eerste zwangerschap na intake. Daarnaast streeft het model ernaar om dynamisch de kans op een succesvolle geboorte te voorspellen op basis van elke uitkomst van de zwangerschap na de intake.

Bij de ontwikkeling en rapportage van het model is gebruik gemaakt van de TRIPOD-verklaring, die richtlijnen biedt voor het rapporteren van voorspellende modellen, en de PROBAST-tool, die helpt bij het minimaliseren van het risico op bias in het onderzoek. Door belanghebbenden, zoals clinici en patiëntenorganisaties voor stellen met vruchtbaarheidsproblemen, te betrekken, wordt gestreefd naar een solide basis voor het gebruik van dit model in de toekomst.

Het uiteindelijke doel van dit model is om op maat gemaakte en individuele schattingen te bieden van de kans op een succesvolle zwangerschap. Het model zal voortdurend worden bijgewerkt op basis van het nieuwste wetenschappelijke bewijs, zodat het de best mogelijke geïndividualiseerde counseling kan blijven bieden. Het is belangrijk om patiënten en clinici actief te betrekken bij de ontwikkeling en implementatie van dit predictiemodel om ervoor te zorgen dat het waardevol en bruikbaar is in de klinische praktijk.

CONCLUSIES EN BLIK OP DE TOEKOMST

Dit proefschrift heeft waardevol inzicht geboden in de dagelijkse praktijk van herhaalde miskramen zorg en counseling. Het onderzoek toont aan dat er ruimte is voor verbetering in de zorg en begeleiding van stellen met herhaalde miskramen. Het feit dat veel van deze stellen geen identificeerbare risicofactoren lijken te hebben, kan ontmoedigend zijn. Het is belangrijk om de variatie in praktijken en de redenen daarachter te erkennen en te streven naar effectieve, op bewijs gebaseerde zorg die het principe van "niet schaden" handhaaft.

De focus van toekomstig onderzoek moet liggen op het verbeteren van de kwaliteit van zorg en het beantwoorden van de meest dringende vragen in de herhaalde miskramen zorg. Begeleiding bij toekomstige zwangerschappen speelt een cruciale rol, zelfs in afwezigheid van effectieve behandelingsopties die de zwangerschapskansen verbeteren. Het is essentieel om goed ontwikkelde en gevalideerde predictiemodellen te gebruiken om paren te helpen weloverwogen beslissingen te nemen over verdere zwangerschapspogingen.

Samenwerking en verbinding tussen samenlevingen, onderzoekers en clinici over de hele wereld is van groot belang om de zorg voor herhaalde miskramen te optimaliseren. Met geavanceerde technologieën en wereldwijde connectiviteit kunnen we een nieuwe dimensie geven aan het onderzoek naar herhaalde miskramen en uiteindelijk de mysteries ervan ontrafelen. Door hoogwaardig onderzoek met een grote impact te bevorderen, kunnen we streven naar betere zorg en counseling voor stellen met herhaalde miskramen.

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ABBREVIATIONS

ACA, anticardiolipin antibodies

AGREE, appraisal of guidelines for research & evaluation

FI30, fertility index truncated at 30 years old

ANA, antinuclear antibodies

APC, activated protein c

APS, antiphospholipid syndrome

APTT, activated partial thromboplastin time

ART, assisted reproductive technology

ASRM, American society for reproductive medicine

AT III, antithrombin 3

AUMC, Amsterdam universitair medisch centrum

aβ2GPI, anti-β2-glycoprotein 1

BMI, body mass index

C-statistic, concordance statistic

CBS, central bureau statistiek

CI, confidence interval

CMV, cytomegalovirus

CBRC, cross-border reproductive care

HbA1C, haemoglobin A1c

HCG, human chorionic gonadotropin

HLA, human leukocyte antigen

HSG, hysterosalpingography

INR, international normalised ratio

HCG, human chorionic gonadotropin

IVF, in vitro fertilisation

IVIG, intravenous immunoglobulin

LAC, lupus anticoagulant

LH, luteinizing hormone

LMWH, low molecular weight heparin

LUMC, Leiden universitair medisch centrum

MRI, magnetic resonance imaging

N, number

NK, natural killer

NSAID, nonsteroidal anti-inflammatory drug

NVOG, Nederlandse vereniging voor obstetrie en gynaecologie

OPAL, on pregnancy after losses

OR, odds ratio

PCOS, polycystic ovary syndrome

PGT, preimplantation genetic testing

PRISMA, preferred reporting items for systematic reviews and metanalyses

PROBAST, prediction model risk of bias assessment tool

PT, prothrombin time

PUL, pregnancy of unknown location

RCOG, royal college of obstetricians and gynaecologists

RCT, randomized controlled trial

REMI, recurrent miscarriage

ROB, risk of bias

ROC, receiver operating characteristic

RPL, recurrent pregnancy loss

SD, standard deviation

SIS, saline infusion sonohysterography

TLC, tender loving care

TPO, thyroid peroxidase

TRIPOD, transparent reporting of a multivariable prediction model for individual prognosis or diagnosis

TSH, thyroid stimulating hormone

VTE, venous thromboembolism

CONTRIBUTING AUTHORS

K.W.M. Bloemenkamp, Department of Obstetrics and Gynaecology, University Medical Centre Utrecht, Utrecht, The Netherlands.

E. van den Boogaard, Department of Obstetrics and Gynaecology, Centre for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Amsterdam University Medical Centre, Amsterdam, the Netherlands.

S.W.M. Dieben, Department of Obstetrics and Gynaecology, Leiden University Medical Centre, Leiden, the Netherlands.

M. Dongen, Department of Obstetrics and Gynaecology, Leiden University Medical Centre, Leiden, The Netherlands.

R. van Eekelen, Centre for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Amsterdam University Medical Center, Amsterdam, Netherlands.

N.A. du Fossé, Department of Obstetrics and Gynaecology, Leiden University Medical Centre, Leiden, the Netherlands.

N. van Geloven, Department of Biomedical Data Sciences, Leiden University Medical Centre, Leiden, The Netherlands.

M. Goddijn, Department of Obstetrics and Gynaecology, Centre for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Amsterdam University Medical Centre, Amsterdam, the Netherlands.

R.P.M.G. Hermens, Scientific Institute for Quality of Healthcare, Radboud University, Nijmegen, the Netherlands.

M.L.P. van der Hoorn, Department of Obstetrics and Gynaecology, Leiden University Medical Centre, Leiden, the Netherlands.

J.A.M. Kremer, Faculty of Medical Sciences, Radboud University, Nijmegen, the Netherlands.

E.E.L.O. Lashley, Department of Obstetrics and Gynaecology, Leiden University Medical Centre, Leiden, the Netherlands.

J.M.M. van Lith, Department of Obstetrics and Gynaecology, Leiden University Medical Centre, Leiden, the Netherlands.

A.G.M.G.J. Mulders, Department of Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam, The Netherlands.

E. Rijgersberg, Centre for Reproductive Medicine, A Amsterdam Reproduction and Development Research Institute, Amsterdam University Medical Centre, Amsterdam, the Netherlands.

M.A.J. Smits, Department of Obstetrics and Gynaecology, Centre for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Amsterdam University Medical Centre, Amsterdam, the Netherlands.

H.J. Verburg, Department of Obstetrics and Gynaecology, Leiden University Medical Centre, Leiden, the Netherlands.

H.R. Verhoeve, Department of Obstetrics and Gynaecology, Onze Lieve Vrouwe Gasthuis, Amsterdam, the Netherlands.

N. Vermeulen, European Society of Human Reproduction and Embryology, Strombeek-Bever, Belgium.

J. Visser, Department of Obstetrics and Gynaecology, Amphia Hospital, Breda, The Netherlands.

M. van Wely, Department of Obstetrics and Gynaecology, Centre for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Amsterdam University Medical Centre, Amsterdam, the Netherlands.

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CURRICULUM VITAE

Angelos Youssef werd op 21 oktober 1995 geboren te Amsterdam, waar hij in 2013 zijn eindexamen behaalde aan het Gymnasium Damstede. Tussen 2013 en 2020 studeerde hij geneeskunde aan de Universiteit Leiden. Reeds tijdens zijn studie raakte hij betrokken bij de onderzoeksgroep herhaalde miskramen.

Aansluitend aan zijn studie heeft hij hier vervolg aan gegeven met zijn promotieonderzoek op de afdeling Obstetrie en Gynaecologie van het Leids Universitair Medisch Centrum onder leiding van Prof. Dr. J.M.M. van Lith, Dr. M.L.P. van der Hoorn en Dr. E.E.L.O. Lashley, waarvan dit proefschrift het resultaat is.

Na 2 jaar werkzaam geweest te zijn als arts-assistent gynaecologie in het Haaglanden Medisch Centrum te Den Haag, start hij per 1 januari 2023 met de opleiding tot gynaecoloog in het Groene Hart Ziekenhuis te Gouda.