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

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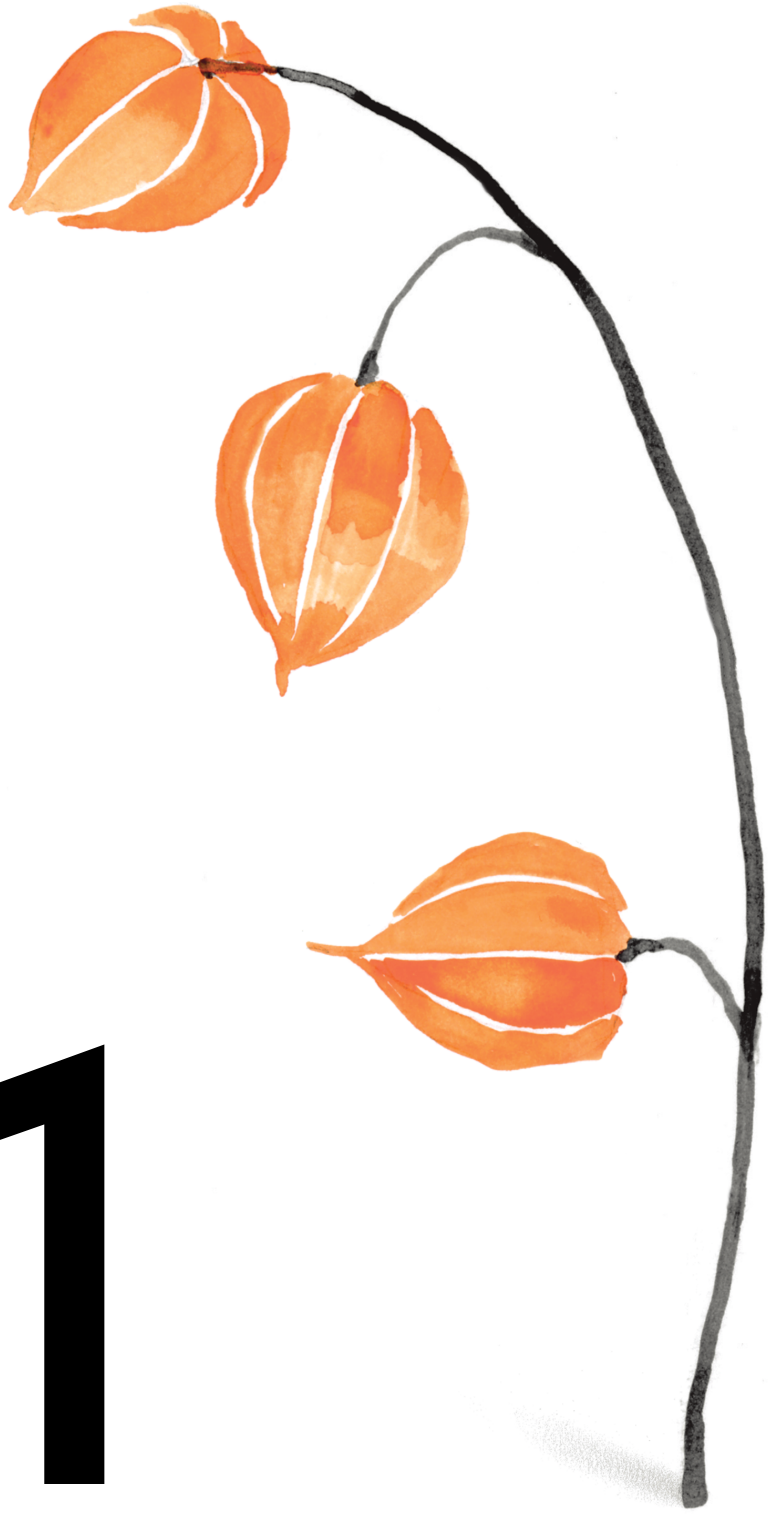

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CHAPTER 1

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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