Unexplained Recurrent Pregnancy Loss: Demographic and Clinical Features

by

Sunaina Sharma

MBBS, International Higher School of Medicine, International University of Kyrgyzstan, 2013

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(REPRODUCTIVE AND DEVELOPMENTAL SCIENCES)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

August 2020

The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, the thesis entitled:

Unexplained Recurrent Pregnancy Loss: Demographic and Clinical Features

submitted by Sunaina Sharma in partial fulfillment of the requirements for

the degree of Master of Science

in Reproductive and Developmental Sciences

Examining Committee:

Mohamed Bedaiwy, Professor, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynaecology, University of British Columbia

Supervisor

Paul Yong, Associate Professor, Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynaecology, University of British Columbia

Supervisory Committee Member

Sarka Lisonkova, Associate Professor, Division of Maternal Fetal Division, Department of Obstetrics and Gynaecology, University of British Columbia.

Supervisory Committee Member

Christian Klausen, Research Associate, Department of Obstetrics and Gynaecology, University of British Columbia

Additional Supervisory Committee Member

Abstract

Unexplained recurrent pregnancy (uRPL) loss is a devastating and challenging condition for couples as well as clinicians. One of the greatest challenges is not being able to identify the baseline etiology of recurrent pregnancy losses (RPL) when all the routine and recommended investigations return normal or negative. Pregnancy losses that are not seen on the ultrasound or are confirmed only based on decreasing urine or serum beta-hCG's are termed as non-visualized pregnancy losses (NVPLs). Routine evaluation of NVPLs is not recommended by the American Society for Reproductive Medicine (ASRM) guidelines.

The first objective of my thesis was to evaluate how different types of miscarriages affect the rates of a successful pregnancy (ongoing pregnancy at or beyond 10 weeks gestation) among the uRPL group. The three different types of miscarriages include visualized pregnancy losses (VPL), NVPL, and a combination of both (mixed). The second objective was to assess the idea of stratifying uRPL women into Type 1 (age ≥35, NVPL) and Type 2 (age<35, mixed, and VPL) groups and further estimate their chances of having a successful pregnancy. The data of 1311 RPL women was used from the BC Women's Hospital, RPL clinic. For the first part, regression analysis was performed, comparing NVPL with VPL groups of uRPL women. The results showed that women with NVPL were more likely to have undergone therapeutic abortions in the past (AOR=3.274, 95% CI=1.425-7.523). The rate of successful pregnancy was lower among women in the NVPL group as compared to the VPL group (AOR=2.43 and 95% CI=1.33-4.42). In the second study, I found that only 8.2% of uRPL women were categorized as Type 1 and 32.3% as Type 2. The rate of successful pregnancy among Type 1 was 31.2% and 52.6% among Type 2 ($p<0.001$). In conclusion, I observed a significant number of NVPLs among uRPL women. Women who had only NVPLs were more likely to experience a miscarriage at <10 weeks' gestation

compared with women with VPLs. Stratification of uRPL women into Type 1 and Type 2 groups might not be helpful and all the uRPL women should be managed irrespective of their age.

Lay Summary

‘Unexplained recurrent pregnancy loss’ is the term used for women experiencing repeated miscarriages (≥ 2) when their laboratory tests and evaluations done by the gynecologist turn out to be normal. According to clinical guidelines, doctors do not investigate or treat women experiencing pregnancy loss based on negative urine/blood test where the pregnancy is not confirmed by ultrasound. These are termed as pre-clinical pregnancy losses. The first part of my thesis focused on the pre-clinical unexplained recurrent pregnancy loss group of women. I found that a significant number of these women were present in our clinic population and were more likely to suffer from miscarriages as compared to the clinically confirmed losses. In the second part of my thesis, I found that both older and younger women are likely to suffer unexplained recurrent pregnancy losses and they should be managed as early as possible in pregnancy.

Preface

Data for this research was obtained from the database of women reported to British Columbia's Women's Hospital (BC Women's Hospital: BCWH), RPL Clinic (a tertiary referral center), Vancouver, British Columbia, from January 2011 to August 2017.

The development of both the cohort studies in this research project was performed under the supervision of Dr. Mohamed Bedaiwy.

For Aim 1 and Aim 2, I reviewed some of the RPL patient charts for finalizing the preliminary data and sample size estimation. I extracted the data from the Research Electronic Data Capture (REDCap) data management platform at BC Children's Research Institute (BCCHRI) and performed the statistical analysis. All statistical analysis, creation of tables and figures were done by me with the guidance of Drs. Mohamed Bedaiwy and Sarka Lisonkova. I also contributed by creating RPL patient intake forms for the clinic that included the patient's personal, gynecological, and medical history followed by the patient's family history and partner's history. I created separate forms that included detailed questions regarding the history of previous pregnancies and the information about the outcome of each successful pregnancy as well.

The study was put into action after the approval by the University of British Columbia ethics board (Approval number: H13-03306). This thesis was revised by Dr. Mohamed Bedaiwy, Dr. Sarka Lisonkova, and Dr. Paul Yong. Further, it was edited entirely by me based on the suggestions provided by my committee members.

Manuscripts will be prepared for the future publication based on the results of Chapters 2 and 3. The manuscripts will include the introduction, methods, results, and discussion sections of Chapters 1, 2, 3 and 4.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

aCL = anticardiolipin antibody

AOR = adjusted odds ratio

aPL = antiphospholipid antibodies

APS = antiphospholipid syndrome

ART = artificial reproductive technology

ASRM = American Society for Reproductive Medicine

BCCHRI = BC Children's Hospital Research Institute

BCCHRI = BC Children's Research Institute

BCWH = BC Women's Hospital

BMI = body mass index

BPL = biochemical pregnancy loss

CBC = complete blood count

CGH = comparative genomic hybridization

CI = confidence interval

CMA = chromosomal microarray analysis

CMV = cytomegalo virus

CNV = copy number variants

CT = computed tomography scan

EP = ectopic pregnancy

ESHRE = European Society for Human Reproduction and Embryology

FISH = fluorescence in-situ hybridization

G-CSF = granulocyte colony stimulating factor

HCG = human chorionic gonadotropin hormone

ICSI = intracytoplasmic sperm injection

IUD = intrauterine device

IVF = in-vitro fertilization

IvIg = intravenous immunoglobulin

LA = lupus anticoagulant

LBR = live birth rate

LIT = lymphocyte immunization therapy

LMP = last month period

LMWH = low molecular weight heparin

Mb = megabase

MRI = magnetic resonance imaging

NK = natural killer cells

NPV = negative predictive value

NTD = neural tube defect

NVPL = non-visualized pregnancy loss

PCOS = polycystic ovarian syndrome

PGD = preimplantation genetic diagnosis

PGS = preimplantation genetic screening

PGT = preimplantation genetic testing

POC = products of conception

PPV = positive predictive value

PUL = pregnancy of unknown location

RC = reference category

RCOG = Royal College of Obstetricians and Gynaecologists

RCT = randomized controlled trial

REDcap = research electronic data capture database

REPL = recurrent early pregnancy loss

RM = recurrent miscarriage

RPL = recurrent pregnancy loss

RR = relative risk

SNP = single nucleotide polymorphism

TLC = tender loving care

TPO-Ab = thyroid peroxidase antibody

TSH = thyroid stimulating hormone

TVS = transvaginal ultrasound

uRPL = unexplained recurrent pregnancy loss

USG = ultrasonography

VOUS = variant of unknown origin

VPL = visualized pregnancy loss

Acknowledgments

First and foremost, I would like to thank my supervisor and a great mentor, Dr. Mohamed Bedaiwy for giving me an opportunity to work under his supervision. I am very grateful for his support, advice, and for all the incredible learning opportunities that I have got being his student.

I would also like to thank my supervisory committee members Dr. Paul Yong, Dr.Sarka Lisonkova, and committee chair Dr.Dan Rurak for their continuous guidance and helpful feedback.

Special gratitude to all the patients at the BC Women's Hospital, who made the largest contribution to this research.

A heartfelt thanks to my colleagues in Dr.Bedaiwy's lab and the BC Women's hospital for all their support.

With my utmost appreciation, I would like to thank my wonderful parents Karnail Singh and Vandana, for being my biggest support emotionally thorough out this endeavor.
My sister Nancy, who listens to me empathetically and encourages me through the stressful times. Finally, my brother Prawal, for always cheering me up and motivating me to stay mindful and healthy.

Lastly, thank you to all my lovely friends for being compassionate throughout this journey.

Dedication

This Thesis work is dedicated to

all those women and their families

who are suffering from

Recurrent Pregnancy Loss.

Chapter 1: Introduction

1.1 Pregnancy loss

Pregnancy loss is a spontaneous end of a pregnancy that was previously confirmed by a minimum of two positive beta hCG assays in the serum or urine [1]. A combination of high-resolution transvaginal ultrasound and sensitive urine/serum beta-hCG measurements is the best for the diagnosis of pregnancy in very early gestation [1].

1.2 Terminology

Based on this, a pregnancy loss can be categorized under different terminologies as recommended by the ESHRE guidelines. A pregnancy loss that has not been confirmed with ultrasound or histopathologic examination is termed as non-visualized pregnancy loss (NVPL). A pregnancy based on serum/urine beta hCG resulting in further decrease/negative beta-hCG is termed as biochemical pregnancy loss. Term 'pregnancy of unknown location' (PUL) is used when a pregnancy test is positive but transvaginal ultrasonographic evaluation fails to localize the current pregnancy [2]. After evaluation and confirmation of PUL, we can further categorize it into an ectopic pregnancy, intrauterine pregnancy, resolved pregnancy, or treated PUL [2].

An intrauterine pregnancy that has been confirmed with ultrasound examination and has histological evidence is termed a clinical miscarriage. Further, these clinical miscarriages are subdivided into early and late clinical pregnancy losses. Early pregnancy loss is defined as loss of a clinical pregnancy before 10 weeks of gestation or if by any chance gestational age is unknown, then it is determined by loss of an embryo/fetus of <400 g [3]. Early pregnancy losses occur in 15%-20% of pregnancies and their prevalence increases with advanced maternal age [4,5]. Kolte et al., 2014 have very well summarized all the terminologies in a table (Table 1.1) [1].

Table 1.1 Summary of terminologies of all the classified pregnancy losses. Adapted from [1]

Terminology for classifying pregnancy failure prior to viability for research purposes.		
Term	Description of pregnancy loss and clinical or ultrasound findings	References
Pregnancy loss	Spontaneous pregnancy demise	
Early Pregnancy loss	Spontaneous pregnancy demise before 10 weeks of gestational age(before 8th developmental week)	
Non-visualized pregnancy loss	Spontaneous pregnancy demise based on decreasing serum or urinary β-hCG levels and non-localization on ultrasound, if performed	Kolte et al. (2014)
Biochemical pregnancy loss	Spontaneous pregnancy demise based on decreasing serum or urinary β-hCG levels, without an ultrasound evaluation	Farquharson and Stephenson (2010)
Resolved pregnancy loss of unknown location	Pregnancy demise not visualized on transvaginal ultrasound with resolution of serum β-hCG after expectant management or after uterine evacuation without chorionic villi on histology	Barnhart et al. (2011)
Treated pregnancy loss of unknown location	Pregnancy demise not visualized on transvaginal ultrasound with resolution of serum β-hCG after medical management	Barnhart et al. (2011)
Miscarriage	Intrauterine pregnancy demise confirmed by ultrasound or histology	ASRM Practice Committee (2013)& Stephenson and Kutteh (2007)
Early miscarriage	Intrauterine pregnancy loss <10 weeks size on ultrasound	
Anembryonic (empty sac) miscarriage	Intrauterine pregnancy loss with a gestational sac but without a yolk sac or an embryo on ultrasound	
Yolk sac miscarriage	Intrauterine pregnancy loss with a gestational sac and yolk sac, without an embryo on ultrasound	
Embryonic miscarriage.	Intrauterine pregnancy loss with an embryo without cardiac activity on ultrasound	
Fetal miscarriage.	Pregnancy loss ≥ 10 weeks size with a fetus ($\geq 33mm$) $on\ ultrasound$	Stephenson & Kutteh (2007)
Ectopic pregnancy	Ultrasonic or surgical visualization of a pregnancy outside of the endometrial cavity	Barnhart et al. (2011) Barnhart (2009)

1.3 Epidemiology of pregnancy losses

Many prospective cohort studies have shown that only one-third of conceptions progress to a live birth in women trying to conceive [4,6,7]. 30% of conceptions are lost even prior to implantation and 30% are lost post-implantation i.e. in the third and fourth weeks of gestation. These are categorized as preclinical losses [8]. 70% of conceptions are found to be lost before live birth and the majority of those losses are preclinical. Macklon et al., 2002, and Larsen et al., 2013, have described a pregnancy loss iceberg, showing an overview of the outcome of spontaneous human conceptions (on the 'iceberg', these preclinical losses are shown below the 'waterline') Figure 1.1 [8,9].

15% to 20% of the spontaneous miscarriages occur among healthy couples and of these 2% to 3% result in recurrent spontaneous pregnancy losses [10]. There is a very strong correlation between maternal age and the incidence of miscarriages [11] with the underlying cause suggested as the frequency of aneuploidy in the oocytes [12]. Grande et al., 2012, showed that the risk of miscarriage increases significantly in older women (≥35 years) by 9.5% at the age of 24 to 25 years and up to 76% at age ≥45 years [11].

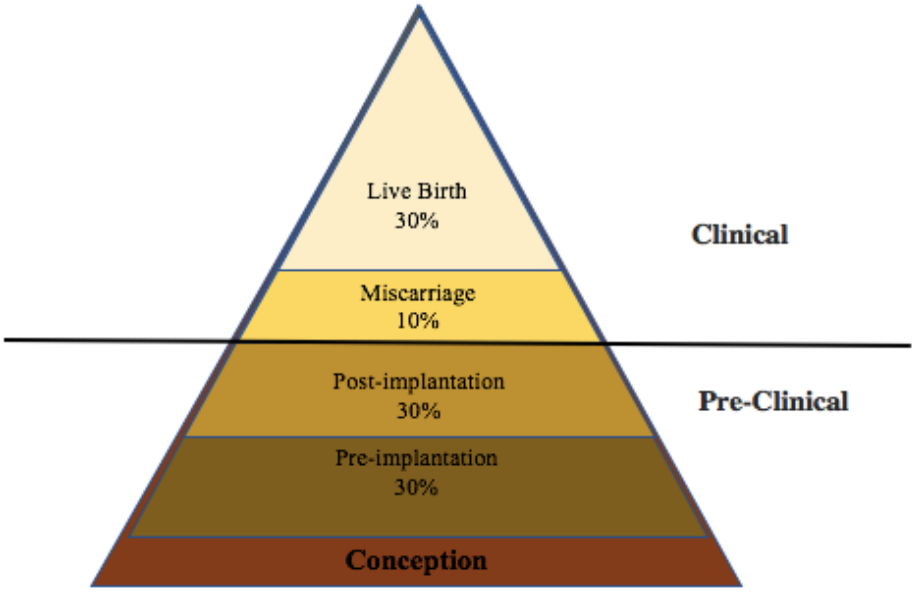


Figure 1.1 The pregnancy loss iceberg showing an overview of the outcome of spontaneous human conceptions. Adapted and edited from [8,9]

1.4 Physiology of implantation

Several processes and factors play a role in a successful implantation process. Within 24 to 48 hours of ovulation, fertilization takes place in the fallopian tube. The zygote (a fertilized ovum) develops to become a morula (a mass of 12 to 16 cells) enclosed by an outer non-adhesive covering called zona pellucida. Further growth of the morula results in a blastocyst [13,14]. The outer

surface of blastocyst gives rise to the trophoblast which becomes the placenta and its inner cell mass gives rise to the embryo. After entering the uterine cavity, within 72 hours, the embryo breaks its outer surface exposing the multinucleated trophoblast layer. Implantation takes place within 6 to 7 days after fertilization. The blastocyst is completely implanted in the uterine cavity by the 10th day after fertilization [13-17]. Within this large fluid-filled gestational sac, the embryo is located in two sacs called the amnionic and chorionic sacs. For pathological evaluation of the products of conception, the components of the gestational sac that are examined include chorionic villi, decidua, implantation site, endometrial vessels and glands [18-21].

1.5 Pathogenesis of early pregnancy loss

The key factors marking successful implantation rely on the uterine receptivity and the blastocyst activation. Uterine receptivity is the maturation phase of the endometrium during which the blastocyst can implant successfully [22]. Implantation in the uterus depends on processes like the maturation of the surface epithelium, endometrial secretion composition, count, and types of immunocompetent maternal cells and receptors on the trophoblast. Studies have shown that two-thirds of the early pregnancy losses are due to defective placentation as a result of decreased extravillous cytotrophoblast invasion of the spiral arteries. This further leads to trophoblastic oxidative damage and degeneration of placenta [13,15,16,22,23]. Both implantation and placentation are key processes in early pregnancy and knowing how complex both the processes are, there could be many possible mechanisms involved in their pathogenesis. About 25% to 40% of pregnancy losses occur after implantation. Most of these are a result of genetic abnormalities but the exact baseline cause is not known [24].

1.6 Diagnosis of pregnancy loss

Pregnancy loss can be diagnosed by ultrasound or visually by the presence of bleeding or evident expulsion of an embryo or fetus. Pathological evaluation of the expelled embryonic or fetal tissue can help to identify possible causes of pregnancy loss. In pregnant women with vaginal bleeding but no passage of tissues, an ultrasonographic evaluation is recommended for the further evaluation [25-31]. The Royal College of Obstetricians and Gynecologists (RCOG) specifies a diagnosis of a 'failed pregnancy' if there is no yolk or fetus present (empty gestational sac > 20 mm) or crown-rump length of not more than 7 mm with absent fetal heart activity. In women with indefinite findings, sequential assessment of serum beta-hCG is the best diagnostic tool to determine a miscarriage [32]. Doubilet et al., 2013, very well described the ultrasound diagnostic criteria and guidelines for detecting the early pregnancy in the most accurate way (Table 1.2) [29].

Table 1.2 Clinical guidelines for transvaginal ultrasonographic diagnosis of pregnancy failure in women with an intrauterine pregnancy of ambiguous viability [29]

Findings diagnostic of pregnancy failure	Findings suspicious for, but not diagnostic of, pregnancy failure
Crown–rump length of ≥7 mm and no heartbeat	Crown–rump length of <7 mm and no heartbeat
Mean sac diameter of ≥25 mm and no embryo	Mean sac diameter of 16–24 mm and no embryo
Absence of embryo with heartbeat ≥2 wk after a scan that showed a gestational sac without a yolk sac	Absence of embryo with heartbeat 7–13 days after a scan that showed a gestational sac without a yolk sac
Absence of embryo with heartbeat ≥11 days after a scan that showed a gestational sac with a yolk sac	Absence of embryo with heartbeat 7–10 days after a scan that showed a gestational sac with a yolk sac Absence of embryo ≥6 wks after last menstrual period Empty amnion (amnion seen adjacent to the yolk sac, with no visible embryo) Enlarged yolk sac (>7 mm) Small gestational sac in relation to the size of the embryo (<5 mm difference between mean sac diameter and crown-rump length)

1.7 Recurrent pregnancy loss

1.7.1 Definitions

Recurrent pregnancy loss (RPL) is a very traumatic condition for the couples and a very challenging situation for clinicians due to its complex definitions and variations in available diagnostic tools worldwide. Its definition has always been controversial. According to the RCOG, RPL is defined as three consecutive pregnancy losses, before 24 weeks' gestation. However, according to the American Society for Reproductive Medicine (ASRM), it is defined as two or more clinical pregnancy losses (documented by ultrasonography or histopathologic examination), and non-visualized pregnancy losses are not included [1,5]. Another early pregnancy specialist group, the European Society for Human Reproduction and Embryology (ESHRE) suggested the same inclusion of two or more pregnancy losses but permitted including non-consecutive pregnancy losses while the other guidelines focus on including only consecutive pregnancy losses [33,34]. [5,34]According to ESHRE, the term NVPL should only be used in the absence of clinical evidence of pregnancy loss either by ultrasound or by histopathology [35]. For these cases, the diagnosis of pregnancy loss depends on decreasing serum or urinary β hCG and by ultrasounds when the gestational location is not visualized [35]. Table 1.3 demonstrates the different definitions of RPL. RPL is further subdivided into primary, secondary, and tertiary RPL. Women with multiple pregnancy losses without any viable pregnancy are categorized under primary RPL. Secondary RPL is defined as multiple losses in a woman who has had at least one pregnancy going beyond

20 weeks of gestation and tertiary RPL signifies multiple losses occurring between viable pregnancies [1,36].

Table 1.3 Comparing the elements of definitions of RPL according to different International guidelines [37,38].

	ESHRE[a]	ASRM[b]	RCOG[c]
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 the majority are lost prior to 10th week	Up to 24 weeks
Recurrence	2	2	3
Consecutive	Consecutive or non-consecutive	Consecutive	Consecutive

a. European Society for Human Reproduction and Embryology,
b. American Society for Reproductive Medicine**,**
c. The Royal College of Obstetricians and Gynecologists

1.7.2 **Prevalence**

It is very difficult to estimate the absolute prevalence of RPL. In a study done by Stray et al, 1979, a prevalence of 1.4% was reported among women with a history of ≥2 consecutive RPLs[39]. This is a very old study and at that time there were not many diagnostic methods available to detect early pregnancy losses. Another study by Larsen et al., 2013, reported that the prevalence of pure visualized pregnancy losses (confirmed by ultrasound and/ or histology) was 0.8% to 1.4% and they mentioned that by including biochemical losses, the prevalence increases to 2% to 3%[9] **.** In a quite recent questionnaire study of Japanese women aged 35 to 79 years, 0.88% of women had a history of ≥ 3 consecutive RPL [40].

According to ESHRE [33,37], recurrent pregnancy loss affects around 1% to 2% of women whereas, as per ASRM, the incidence of RPL is not less than 5% in women with two or more failed

clinical pregnancies [5]. We strongly believe that the prevalence would increase by a significant number if these studies were repeated.

1.7.3 Causes of recurrent pregnancy loss

1.7.3.1 Genetic factors

50% to 60% of early pregnancy losses are because of genetic abnormalities with parental origin or in the embryo from parents with normal chromosomes [41,42]. The risk of fetal loss rises steeply after the age of 35 years [12], increasing from 9.5% at 20–24 years to 76% at 45 years and older. The relationship between maternal age and the rate of miscarriage is correlated with aneuploidy frequency in oocytes. There is a 10% risk of aneuploidy in the oocytes in the younger women (<35 years). But this risk increases to 50% in the women at around 43 years of age and 100% of oocytes are found to exhibit some aneuploidy in women aged 45 years and older [12,37]. It thus becomes important to differentiate between chromosomal abnormalities in parents and the products of conception. [12,37]

1.7.3.1.1 Parental

Among parental abnormalities, the most common structural chromosomal defects found are balanced translocations that account for 2% to 4% of RPLs; the incidence is 0.7% in the overall population [43,44]. These could either be reciprocal or Robertsonian translocations. Couples with chromosomal defects are at high risk of producing non-viable embryos. Their gametes with unbalanced chromosomal variants could be a cause of RPL. IVF and preimplantation genetic diagnosis (PGD) are alternatives to natural conception for these couples. Along with IVF and PGD, patients may also choose to undergo clinical management by undergoing ultrasound and early

pregnancy assessment to identify abnormalities [45]. Franssen et al, 2006, and Barber et al., 2010, reported a 1.9% incidence of abnormal parental karyotypes in their large retrospective cohort of 20432 patients referred for genetic analysis after RPL [46,47]. Another retrospective study was done by Flynn et al., 2014, where in a cohort of 795 couples with two or more pregnancy losses, 3.5% of couples were found to have chromosomal defects [48].

Maithripala et. al., 2017, performed a retrospective chart review of 2321 couples referred to our tertiary RPL clinic. They reviewed the treatment choices made by couples with structural chromosomal rearrangements. Further, they also looked at the rate of live births among these couples who chose IVF and PGD instead of choosing natural conception and prenatal diagnosis. They found that most of the RPL couples prefer considering the natural conception method instead of IVF or PGD. Also, they did not find any significant difference in the live birth rate among the couples who chose PGD or clinical treatment [45].

1.7.3.1.2 Products of conception

Pregnancies with abnormal or unbalanced translocations mostly result in miscarriage, stillbirth, or live births with the presence of congenital defects. Couples with balanced translocation can either have an abnormal karyotype of their products of conception (POC) or might even have completely normal results. Studies have shown that 25% to 35% of the POC evaluated have unbalanced translocations [4,5,41]. The most common cause of early pregnancy loss (<10 weeks' gestation) is found to be embryonic aneuploidy and around 90% of embryos with chromosomal defects are aborted spontaneously. Structural abnormalities found to be associated with RPL are chromosomal inversions, and insertions [37]. The most frequent abnormalities found in the POC analysis are numerical chromosomal defects like monosomy X, polyploidy, and

trisomy. Among these, trisomy is the most commonly reported chromosomal abnormality among women with advanced age [4,5,41].

1.7.3.2 Autoimmune factors

1.7.3.2.1 Antiphospholipid syndrome (APS)

APS is characterized by the presence of antiphospholipid antibodies (aPL) in the blood of women experiencing RPLs [49]. Antibodies diagnostic of APS include lupus anticoagulant, anticardiolipin antibody, and anti-β2 glycoproteins I. The prevalence of APS in women with RPL is found to be about 15% to 20% [49-52]. aPL impairs the trophoblastic invasion and hampers the secretion of β hCG. Along with initiating syncytiotrophoblast apoptosis, aPL also induce inflammatory responses via complement activation at the maternal-fetal interface further affecting the formation of spiral arteries by targeting the vascular endothelium. This results in various obstetric complications like intrauterine growth restriction, fetal prematurity, and severe preeclampsia [53-55]. With the exception of APL diagnostic antibodies, the clinical assays for aPL antibodies are not standardized and are therefore not recommended for routine screening [5].

1.7.3.2.2 Inherited thrombophilia

Thrombosis of spiral arteries within the placenta affects placental perfusion, which may result in intrauterine growth restriction, severe preeclampsia, placental abruption, or fetal loss. Inherited thrombophilia factors associated with second and third-trimester losses comprise prothrombin gene mutation (G20210A), FVL gene mutation, protein C and S, and antithrombin deficiency [53,56]. The RCOG guidelines recommend that women with the second-trimester

miscarriage should be screened for inherited thrombophilias including factor V Leiden, factor II (prothrombin) gene mutation, and protein S [57].

However, the evidence is less clear and routine screening for inherited thrombophilias is not recommended by ASRM and ESHRE guidelines, unless the patient has a personal history of thrombosis (not related to any previous surgery) or any family history of a first degree relative with a high risk of thrombophilia [5,33] .

1.7.3.3 Anatomical factors

Uterine defects account for up to 10% to 15% of RPL patients [58-60]. Second trimester losses are associated with congenital uterine anomalies along with other obstetric complications including fetal malpresentation, preterm labor, and cesarean delivery [61]. The role of uterine abnormalities in the first trimester is not completely understood but it is recommended to evaluate the uterine cavity in RPL patients to rule out any malformations [62]. Congenital malformations of the uterus are due to abnormal development of Mullerian ducts, and the most common type includes septate, arcuate, bicornuate, Didelphis, T-shaped, and unicornuate uterus [63].

Acquired uterine anomalies include intrauterine adhesions, fibroids, and endometrial polyps. Intrauterine adhesions increase the risk of miscarriage due to the inadequate or damaged endometrial environment that further causes Asherman's syndrome. Among uterine fibroids, submucous fibroids are the most common and destructive as they hinder the implantation and affect the endometrial receptivity [63]. The association between RPL and uterine fibroids is still not well studied.

1.7.3.4 Endocrine factors

Thyroid hormones are very important for the development of a healthy fetus in early pregnancy; the fetus depends completely on maternal thyroid hormone up to 13 weeks' gestation [64]. Vissenberg et al., 2015, mentioned in their review that abnormal thyroid hormone levels and thyroid peroxidase antibodies (TPO-Ab) affect spermatogenesis, folliculogenesis, fertilization, and embryogenesis, and therefore have a significant role as causes of subfertility and pregnancy loss [65].

Autoimmune thyroid disease, frequently reported in women, can lead to certain pregnancy complications such as pregnancy losses, preterm labor, infertility, and poor cognitive outcomes in offspring [66-68]. Thyroid-related negative outcomes could be prevented by treating both overt hypothyroidism (TSH > 10 mIU/L) and subclinical hypothyroidism (TSH between 4 to 10 mIU/L)[68,69]. During the first trimester, the demand for thyroid hormone in the maternal blood increases with the rapid increase of beta-hCG and if there is not enough thyroid reserve in the gland, it might lead to pregnancy complications [71,72].

Prolactin is another important endocrine hormone and its abnormal increase (hyperprolactinemia) may be associated with RPL as a result of the alterations in the hypothalamic-pituitary axis [33 73]. Prolactin testing is not routinely recommended unless the patient presents with symptoms of hyperprolactinemia (oligomenorrhea or amenorrhea) [33].

1.7.3.5 Infection

In women with sporadic miscarriages, the vaginal and cervical cultures frequently contain pathogens such as Chlamydia, Listeria monocytogenes, Mycoplasma hominis, Ureaplasma urealyticum, Toxoplasma Gondii, Rubella, Herpes simplex, Cytomegalovirus and others that are

less frequent [31]. There is not enough evidence that these pathogens can cause RPL and routine screening for infectious pathogens in RPL patients is not recommended [5].

On the other hand, chronic endometritis, even if asymptomatic, is associated with Chlamydia, gonorrhea, and non-sexually transmitted infections including Escherichia coli, Streptococcus, Staphylococcus, and Enterococcus faecalis. Endometritis can also occur due to retained POC (incomplete miscarriage) or placental tissue. A study by McQueen et al., 2014, showed a high prevalence (9%) of chronic endometritis in a cohort of RPL women; the proportion of chronic endometritis was 7% in women with REPL (two or more losses < 10 weeks), 14% in women with at least one loss > 10 weeks, and 11% in the two groups combined [70]. This may indicate a high prevalence of chronic endometritis in women with pregnancy loss but there is not enough evidence to support a routine endometrial biopsy for patients with RPL [5].

1.7.3.6 Others

Obesity is associated with certain pregnancy complications, cardiovascular disease, endometrial cancer, and endocrine disorders such as thyroid diseases, diabetes, and PCOS. Boots et. al., 2014., performed a large group study on obese and non-obese REPL women to determine the frequency of euploid miscarriages. Their results showed that the obese women with a history of REPL presented with a higher number of euploid miscarriages, and were at increased risk of experiencing subsequent miscarriages [71].

Some male factors can contribute to RPL. A positive association between abnormal sperm morphology and RPL has been suggested by some studies; however, sperm testing is still not included in the recommended routing testing for RPL couples [5,72-75] .

Lifestyle and environmental factors that might contribute to the etiology of RPL include cigarette smoking, and the use of alcohol (3 to 5 drinks per week), cocaine, and/or caffeine (>3 cups of coffee per day). Staying healthy psychologically is also important during pregnancy [76,77]. RPL affects the emotional and mental wellbeing of the couple, and patients are therefore advised to seek psychological care while going through RPL investigations and testing [5].

1.7.4 **Evaluation of RPL**

According to ASRM, RPL evaluation should be initiated after the second clinical loss [5,78]. This evaluation includes two sets of tests:

- Evidence-based tests include parental karyotyping, uterine anatomical examination, tests to check the presence of lupus anticoagulant and anticardiolipin antibodies, factor V Leiden, and thyroid stimulating hormone [79,80].
- Investigative tests checking mid-luteal progesterone hormone levels, serum prolactin, antiphosphotidyl serine antibodies, factor II gene mutation (prothrombin), and cervical cultures to rule out infection [34].

These diagnostic tests can be very expensive and time-consuming. Bernardi et al, 2012, examined selective RPL evaluations and whether this could be less expensive in comparison to the routine investigations. They found that the selective evaluation saved 1155 USD in comparison to the comprehensive RPL evaluation [81]. Jaslow et al., 2010, and Foyouzi et al., 2012, also supported this idea [82,83]. Jaslow et al., 2010, found that in a cohort of 1020 RPL couples, 40% of evaluations resulted in at least one abnormal diagnostic test result. They found that the frequency of patients presenting with one or more abnormal test results was similar to (a) women experiencing two, three, or more pregnancy losses (b) women who had previous live births and (c) woman who never

had the previous history of live births. Among 403 patients, 12.9% showed abnormal results for two evidence-based tests and 0.5% had abnormal results for a combination of three tests: lupus anticoagulant, anti-cardiolipin antibodies, and uterine anatomy and parental genetics. This study group recommended that testing should be initiated after two or more pregnancy losses instead of making the patient wait for her third miscarriage. The patients presenting with abnormal evidence-based and investigative diagnostic tests did not differ significantly among women with a distinct number of pregnancy losses in their cohort [82].

The routine RPL evaluation starts with a detailed patient history that includes questions about age, obstetric history, history of pregnancy losses, alcohol intake, or use of any drug. Physical examination starts with a measurement of body mass index (BMI), palpation of inguinal lymph nodes, presence of hirsutism, galactorrhea (in case of endocrinopathy), malar flushing, and telangiectasia (representing presence of autoimmune disease). Pelvic organs are evaluated to rule out any malformations.

1.7.4.1 Genetics

1.7.4.1.1 Parental karyotyping

According to ASRM, parental karyotyping is indicated to detect chromosomal abnormalities including balanced translocations (Robertsonian and reciprocal), inversions and deletions. The results of these tests can help in further advising these patients about preimplantation genetic testing (PGT), chorionic villous sampling, and amniocentesis. These may help further in establishing a prognosis for the following pregnancy [84].

1.7.4.1.2 POC evaluation

Cytogenetic analysis of POC by various new technologies has improved our understanding of the causes of unexplained recurrent pregnancy losses. Information obtained by using these techniques is important diagnostically and prognostically with respect to recurrent miscarriages. Maternal cell contamination and failure of cell culture have been major concerns of previous techniques used in POC analysis, including routine karyotyping, G-banded karyotyping, fluorescence in-situ hybridization (FISH), and cell culture [84].

Genomic microarrays are an important diagnostic method. Chromosomal microarray analysis (CMA) is the first-line diagnostic tool used for detecting fetal chromosomal or congenital abnormalities. Single nucleotide polymorphism (SNP) microarray and comparative genomic hybridization (CGH) are two different types of microarrays. Among both these types, Polymorphic markers (SNP) based CMA have been used by various studies for the POC evaluation [85-87].

The use of SNP microarray for the POC analysis have shown to increase the rate of aneuploidy detection [88-90]. The most recent ESHRE guidelines recommended the use of array comparative genomic hybridization (CGH) in place of routine karyotyping [33].

Chromosomal microarray analysis can detect unbalanced copy number variants (CNVs) below 1 Mb resolution whereas the regular conventional karyotype can only identify until 5 Mb. The high resolution helps to detect submicroscopic rearrangements that are indicative of unbalanced translocations in POC.

The drawbacks of using microarray analysis are that it may fail to detect maternal cell contamination and that it does not identify balanced translocations, although, there is not much evidence if these balanced translocations are related to pregnancy loss [91]. A systematic review and meta-analysis by Dhillon et al., 2013 [92], demonstrated that CMA identified a significantly greater

number of chromosomal errors compared with traditional karyotyping, although some of the chromosomal abnormalities were a variant of unknown origin (VOUS). Another study by Popescu et al., 2018, used CMA along with the standard ASRM RPL workup evaluation among women with a second pregnancy loss. A definitive cause was identified in 95% (95/100) of the RPL patients. Their results also suggested a 50% cost saving to the health care system and patient by using initial 24-chromosomal microarray analysis of the miscarriage tissue[93]. As for the genetic analysis, CGH is so far considered the gold standard test [94].

1.7.4.2 Anatomical evaluation

A uterine anomaly can be detected using a three-dimensional (3D) sonohysterography, which can detect all anomalies, except for unicornuate uterus with non-connecting horns. It is considered the best diagnostic tool and one of the most cost-effective methods used in identifying uterine malformations [5]. Other diagnostic methods that are not routinely recommended include hysteroscopy, hysterosalpingography, CT, and MRI.

1.7.4.3 Endocrinological evaluation

The most common endocrinological disorders include thyroid dysfunction, diabetes mellitus, and hyperprolactinemia. ASRM does not recommend routine testing for T4 or anti-thyroid antibodies as long as the patient has thyroid-stimulating hormone (TSH) levels within a normal range [95].

TSH levels above 2.5 mIU/L with or without the presence of thyroid antibodies increase the risk of pregnancy complications [96].

To rule out hyperprolactinemia and glucose intolerance, testing for serum prolactin levels, fasting blood glucose level, and hemoglobin A1c are recommended by ASRM guidelines [5,97].

1.7.4.4 Autoimmune factors

The most common tests for autoimmune factors in RPL patients are anticardiolipin antibody (aCL), lupus anticoagulant (LA), and anti B2 glycoprotein I [5].

1.7.5 Treatment options for RPL

Several treatment methods are available for managing RPL.

Hachem et al.,2017, summarized the etiologies of RPL, tests for diagnosis, and various treatment options (Table 1.4) [98].

Table 1.4 Etiologies of RPL, recommended tests for diagnosis and treatment options. Adapted from [98]

Etiology	Tests for diagnosis	Treatment options
Uterine factor	3D ultrasonography, sonohysterography, hysterosalpingography, hysteroscopy	Hysteroscopic resection of septum,
	Magnetic resonance imaging	Myomectomy, hysteroscopic removal of polyps Adhesiolysis
APS	aCL, Anti-Beta2GP1, lupus-anticoagulant	Heparin + Aspirin
Endocrine abnormality	Thyroid stimulating hormone, prolactin	Levothyroxine, Bromocriptine, Diabetes
	fasting glucose or HbA1C	control (metformin, weight loss, nutrition)
Genetic	Karyotype of products of conception, Parental Karyotype	Genetic counselling, Preimplantation genetic diagnosis for a balanced translocation
Environmental factors	Screen for smoking, drug use, excessive alcohol and caffeine	Eliminate environmental factors
Psychological		Psychological support
Luteal phase deficiency	Mid luteal phase progesterone	Progesterone supplements
	endometrial biopsy	
Chronic endometritis	Endometrial biopsy	Antibiotic supplements
Other infections	Cultures	Appropriate treatment
Male factor	DNA fragmentation test on sperm	Lifestyle modifications, multivitamins, donor sperm
Unexplained		Progesterone supplement (no consensus) Immunomodulating treatments (no consensus) and Preimplantation Genetic Screening (no consensus)

1.8 Unexplained recurrent pregnancy loss

Experiencing or dealing with unexplained recurrent pregnancy loss (uRPL) is very disconcerting for couples and clinicians as well. After a large variety of investigative tests and evaluation, the causes of RPL are identified in approximately 40% of the couples; when adding new investigative testing including 23 chromosome microarray analysis, another 10% to 20% of

RPL causes are identified [82]. Therefore, with the combined thorough POC analysis and evaluation of genetics, anatomic, autoimmune and endocrine parental tests, about 50% to 60% of couples can be provided with an explanation for their pregnancy losses [82,99]. However, 40% to 50% of couples will remain without any answers.

Unexplained recurrent pregnancy loss (uRPL) is considered a final diagnosis, if: (a) a complete genetic, endocrine anatomic and immune evaluation performed for the RPL patients shows negative or normal findings [100], (b) chromosomal analysis of the POC repeatedly is normal; and (c) all other lifestyle factors like alcohol consumption, smoking, maternal obesity are ruled out as a cause of RPL [101].

Approximately 50% of women with RPL are known to suffer from uRPL [102,103]. Several possible causes have been studied by various researchers and several treatments have been tried and proposed by the clinical guidelines for the treatment of uRPL.

In general, we assume that for women experiencing uRPL, the prognosis is good with respect to future pregnancies and there is no need for any major medical or surgical treatment. Some of the clinical trials have reported that there was a 50% decrease in the number of pregnancy losses when tender loving care (TLC) was provided to the women [104,105]. In a study done by Saravelos and Li, 2012, it was suggested that uRPL women still have a better prognosis even if they do not experience TLC. The reason behind this is that a certain number of uRPL women are completely healthy and experience pregnancy losses purely due to chance alone, completely similar to women in the general population. A cohort of 844 uRPL who had not undergone any treatment was analyzed. It was reported that among these women, the rate of pregnancy loss was 14% to 26% similar to the 12% to 25% rate occurring in (age between 25 to 39 years) the general population[106].

There is still a proportion of uRPL women who will continue to have pregnancy losses due to an unidentified abnormality and not just by chance.

Saravelos and Li, 2012, examined the differences between the uRPL women who experienced pregnancy losses by chance and the uRPL women who had an unidentified abnormality. They stratified these women into Type 1 and Type 2 groups respectively. Type 1 group uRPL women were the older women (age >35 years) with less than three pregnancy losses including biochemical pregnancy losses. This group was assumed to have abnormal karyotypes in the POC. Type 2 group of uRPL women were younger women (age < 35 years) with more than three pregnancy losses including clinical losses only. This group was supposed to have unidentified pathology.

1.8.1 **Potential causes of unexplained recurrent pregnancy loss (uRPL)**

1.8.1.1 Chance

It has been demonstrated that several women experience RPL by chance [107]. These women do not have any underlying abnormality that could be identified by any routine investigative tests. These routine tests often do not include an examination of abnormal products of conception, and some clinicians do not perform POC testing before referring these women to specialists with a diagnosis of uRPL. Such women are considered to have a good prognosis with regard to their future pregnancy and treatments with intravenous immunoglobulin (IVIg), aspirin, or heparin have not been shown to improve their outcomes.

However, some women suffer from uRPL because of an undetected pathology. These women are likely to be young and have a large number of pregnancy losses [106].

1.8.1.2 Oocyte

Increasing maternal age is related to decreased oocyte quantity and quality. Along with aneuploidy related to increased age, ovarian aging is also one of the major concerns. One prior study described oocyte donations from women with uRPL and found that these women had a weak ovarian hyperstimulation response [108]. It is still not clear if the underlying cause was due to age-related oocyte factors or due to maternal origin.

1.8.1.3 Sperm

Sperm quality is an important fertility factor but its role in uRPL is still being debated. There are various sperm-related factors that could affect the fertility process: morphology, function, concentration, DNA fragmentation, oxidative stress, and Y chromosome deletions [109]. A study by Robinson et al., 2012, suggested that DNA can be damaged during the process of sperm injection in assisted conceptions. They advocated for using safe techniques to transfer the sperm to potentially reduce the risk of pregnancy loss [110].

1.8.1.4 Embryo

The future prognosis for women with uRPL can be improved by reducing the risk of aneuploidy by using techniques including assisted reproductive technology (ART), and preimplantation genetic screening (PGS) of the embryo. Musters et al., 2011, reported that PGS decreased the frequency of pregnancy loss. They also recommended further RCTs in women with uRPL to confirm their results [111].

1.8.1.5 Endometrium

Endometrium plays an important role in the selective process of embryo implantation and decidualization. One of the factors that has been found to affect the process of implantation in women with RPL is an increased level of preimplantation cytokines. This contributes to super-fertility leading to the implantation of poor-quality embryos [112,113]. One prior study focusing on embryo-endometrial interactions stipulated that human cell migratory activity is affected greatly by poor-quality embryos and this further determines the success of pregnancy [114]. Impaired decidualization of endometrial cells is one of the causes of RPL. Coulam in her review mentioned that there is a higher prevalence of superfertility among the RPL population associated with impaired decidualization in comparison to the normal population. They also proposed that one of the causes of impaired decidualization is due to the prolonged window of implantation that further increases the chances of superfertility [115] .

1.8.1.6 Other factors

Various systemic factors can contribute to uRPL. Thyroid hormone plays an essential role in the early period of pregnancy until 13 weeks of gestation as the fetus depends exclusively on the mother's thyroid hormone [116]. With the increased levels of beta-hCG in the first trimester, the demand for TSH also rises and unmet demand can lead to hypothyroidism [117,118]. Levothyroxine treatment is usually initiated to avoid hypothyroidism complications in patients who are vulnerable to develop hypothyroidism [117,118]. Prior studies have shown that there is a higher risk of pregnancy loss when TSH levels are between 2.5 to 4 mIU/L and thyroid autoimmunity is also present [69,119]. According to ESHRE, levothyroxine is recommended for women with RPL to get to the target TSH levels below 2.5 mIU/L. However, the American Thyroid Association suggests using

supplements only if women have TSH levels between 2.5 to 4 mIU/L and positive thyroid peroxidase antibody (TPOAb) [64,120]. A study conducted at our RPL Clinic (yet to be published) investigated if thyroid autoimmunity is independently associated with pregnancy loss. This retrospective study showed no correlation between borderline subclinical hypothyroidism (TSH between 2.5 to 4 mIU/L), and thyroid autoimmunity (positive TPOAb). This study suggested that levothyroxine would result in overtreatment without improving pregnancy loss outcomes.

Other potential causes of uRPL include polycystic ovary syndrome, insulin resistance, hyperandrogenism and certain abnormal immune reactions. There are a few retrospective studies that showed a decline in the rate of pregnancy losses after preconception metformin treatment [121-123]. In regard to abnormal immunologic reactions, peripheral and uterine killer cells were found to be one of the causes of the uRPL [124].

1.8.2 Treatment options

1.8.2.1 Preimplantation genetic testing (PGS)

The American Society of Reproductive Medicine recommends expectant management as the current standard of care for patients with unexplained RPL. Numerical chromosomal aberrations (aneuploidy) are diagnosed in 50% of pregnancy losses attributed to fetal chromosomal abnormalities [125]. Consequently, in-vitro fertilization (IVF) and pre-implantation genetic screening (PGS) has been proposed as a method for reducing miscarriage by selecting only euploid embryos for transfer [123]. PGS includes the analysis of all 23 chromosomes with the help of different techniques such as CGH, array CGH (aCGH), FISH, single-nucleotide polymorphism array, massive parallel sequencing and quantitative polymerase chain reaction [98].

The presumptive advantages of using this technology are a shorter time to pregnancy, lower risk of clinical miscarriage (CM) and increased rate of live birth (LB). However, these theoretical advantages were never substantiated in longitudinal prospective studies nor randomized clinical trials comparing IVF and PGS to the current standard of care, expectant management. Few retrospective studies were performed and these showed that IVF/PGS does not significantly increase pregnancy or live birth (LB) rates or decrease miscarriage rates compared with expectant management [128]. To date, PGS has not been shown to be effective in improving LB rate or decreasing CM rate compared with expectant management except in those PGS cycles that complete the transfer of the euploid embryo. Success rates with PGS are limited by the high incidence of cycle cancellation and a higher no transfer rate, particularly in women over 35 years of age. Counseling RPL patients on their treatment options should include not only success rates with PGS per euploid embryo transferred but also LB rate per initiated PGS cycle and the incurred cost. Furthermore, patients who would like to expedite conception should be counseled that PGS may not accelerate time to conception [98,125-127].

1.8.2.2 Lymphocyte immunization therapy (LIT)

The use of LIT in women with RPL increased in the 1980s after a controlled trial conducted by Mowbray et al., 1985, suggesting the beneficial effect of partner lymphocytes immunization [128]. This idea of using LIT arose from the fact that women with RPL are devoid of anti-paternal antibodies. These antibodies usually are required to protect the fetus from the rejection and induction of LIT was proposed to be helpful [129]. However, after several clinical trials, studies concluded that one of the potential adverse outcomes of this particular therapy is the risk of developing neonatal alloimmune thrombocytopenia, red blood cell antibodies production resulting

in erythroblastosis fetalis. Other risk factors include long term hematological malignancies, HIV, and hepatitis transmission [130,131]. Although, Kling et al., 2006, suggested that the use of LIT immunization before conception reduces adverse effects [132]. The ESHRE guidelines do not recommend the use of LIT for the treatment of uRPL [33].

1.8.2.3 Intravenous immunoglobulin (IVIg)

The use of IVIg is proposed to reduce the symptoms of several autoimmune and inflammatory diseases. Egerup et a., 2015,[104,107] in their meta-analysis and systematic review found that IVIg benefited women with secondary RPL with respect to the incidence of live births after the treatment [133]. Another study by Hutton et al., 2007 and Wang et al., 2016, concluded the same results of the positive benefit of IVIg in women with secondary RPL, but not those with primary RPL [131,134] . ESHRE does not recommend IVIg for treatment of either primary or secondary RPL and suggests that more randomized controlled trials should be conducted focusing on the treatment before conception in women with secondary RPL [33].

1.8.2.4 Prednisolone

The use of glucocorticoids has been helpful in treating several autoimmune inflammatory conditions. In women with RPL due to immune causes, prednisolone has been proposed to be potentially beneficial. Gomma et al., 2014, conducted an RCT including 150 women with uRPL. The RCT showed that the incidence of pregnancy beyond 20 weeks gestation was higher among 74 women receiving prednisolone treatment as compared to 76 women who received a placebo (RR 7.63; 95% CI 3.70-15.70) [135].

There was another study done by Laskin et al., 1997, in women with positive antiphospholipid, antinuclear, anti-DNA or anti-lymphocyte antibodies. A very strong dose of prednisolone was given during the entire pregnancy. In the treated group, the rate of live birth was higher (9%) as compared to the control group, however, these women had a higher risk of preterm birth, diabetes, and hypertension [136].

Clinical guidelines do not recommend glucocorticoids as a treatment for women with uRPL or women with known markers of immunological conditions [33].

1.8.2.5 Anticoagulants

Low dose ASA, antiphospholipid antibodies, and heparin have been used to treat RPL in women without antiphospholipid antibodies. Several authors including De Jong et al., 2014, Pasquier et al., 2015, and Schleussner et al., 2015, conducted RCTs of heparin treatment versus placebo, but no benefits of this treatment were found [137-139]. In contrast, an RCT done by Shaaban et al., 2016, found a decrease in the proportion of miscarriages and an increase in live births [140]. Guidelines do not suggest the use of heparin or low dose aspirin for the uRPL women [33] .

1.8.2.6 Folic acid

Low dose folic acid supplementation is usually prescribed to avoid neural tube defects and decreases plasma homocysteine levels. High levels of folic acid have been associated with adverse outcomes including insulin resistance in newborns[141]. ESHRE recommends the use of low dose folic acid pre-conceptionally to avoid NTDs but there is not enough evidence to recommend the use of folic acid to prevent uRPL[33].

1.8.2.7 Progesterone

Several studies have been conducted to see if using progesterone orally and vaginally can be used to treat uRPL [142,143]. Coomarasamy et al., 2015, conducted a multicenter double-blind, placebo-controlled trial among women with uRPL to examine the effect of progesterone on the rate of live births. Women with uRPL were given 400 mg of progesterone vaginal suppositories from the time when they had a positive urinary pregnancy test [143]. This treatment was continued until 12 weeks but no difference in the incidence of live births was documented in the progesterone group in comparison with the placebo group. In a recent meta-analysis by Saccone et al., 2017 [144], a total of 10 trials was combined including the trial by Coomarasamy et al., 2015. In comparison to the women who did not take progesterone, the risk of pregnancy loss among the women who took progesterone was found to be lower and the rate of live birth was higher. However, seven of the included trials were conducted before 1990, and some results may have been flawed due to the lower quality of the RCTs.

The current clinical guidelines do not support vaginal progesterone use for uRPL [33].

1.8.2.8 Intralipid therapy

Intralipid is an emulsion (soybean oil-based) that has been shown to have an effect in decreasing the production of proinflammatory cytokines and NK cell activation. The use of intralipid therapy as a treatment for immune-mediated miscarriages has been less frequently studied [145-147]. Roussev et al., 2008, studied the effect of intralipid therapy and concluded that intralipid therapy has the power to suppress the cytotoxicity caused by NK cells[147]. This therapy was proposed to improve the implantation and pregnancy management in uRPL due to abnormal

NK cells. One RCT by Meng et al., 2015 found that the incidence of live births among women taking intralipid therapy was similar to women taking IVIg [148].

As there is not enough clinical evidence to support this treatment, it is not recommended to use intralipid therapy in RPL or uRPL women [33].

1.8.2.9 Granulocyte colony stimulating factor (G-CSF)

G-CSF is a hematopoietic cytokine that is known to promote trophoblastic growth and development [149]. Based on animal studies, G-CSF has been found to have anti-abortive effects. Studies conducted by Scarpellini and Sbracia, 2009, and Santjohanser et al., 2013, looked into the use of G-CSF in the women with the history of uRPL [150,151]. In the first study, a randomization controlled trial was conducted in women with uRPL. One group was placebo (n=33) and the other group of uRPL women was treated with G-CSF subcutaneously from day six after the ovulation (n=35). Treatment was continued until menstruation or to the end of the 9th gestational week of pregnancy. All 35 women treated with G-CSF became pregnant and 29 live births were reported, while in the placebo group, 16 live births out of 33 pregnancies were noted (p=0.0061) [150].

One study evaluated the effect of G-CSF in women with RPL who had undergone IVF [151]. In their cohort, they strictly included RPL women who had undergone IVF/ICSI. Among these women, the following groups were compared (a) women who were treated with G-CSF (b) women who were treated with other different kinds of medicines and (c) women who had never used any type of medication. The G-CSF group had a pregnancy rate of 47% and a LB rate of 32%. The group that was treated with other medications had a pregnancy rate of 27% and a LB rate of 14%, while the group without any use of medication had a pregnancy rate of 24% and a LB rate of 13% [151]. Several ongoing randomized trials are testing the use of G-CSF in women with RPL.

Barad et al., 2014, did not find any benefits of G-CSF in women with RPL with respect to improving implantation and pregnancy rates after successful IVF. There is insufficient clinical evidence to prove the efficacy of G-CSF in women with uRPL and hence, this treatment is not currently recommended by the clinical guidelines [33].

1.8.2.10 Endometrial scratching

Endometrial scratching has been shown to be associated with the production of cytokines and chemo attractants that are important for the implantation of the embryo. This procedure is performed during the luteal phase before the IVF/ICSI cycle in women who had repeated implantation failures [152]. No clinical trial has been performed to examine the effect of this therapy and therefore it is not recommended as a treatment of uRPL [33].

1.9 Objectives and hypotheses

1.9.1 Study 1: Types of previous miscarriages and rates of successful pregnancy in women with uRPL: a retrospective cohort study.

Objective

To evaluate demographic and clinical characteristics of women with three types of unexplained recurrent pregnancy loss, VPL, NVPL and mixed (both types), and to assess the rates of a successful pregnancy (ongoing pregnancy at or beyond 10 weeks gestation) in women with VPL and NVPL during 12 months after the first visit followed at the RPL clinic between January 1, 2011, and August 31, 2017.

Hypothesis

- Women with various types of uRPL differ with respect to demographic and clinical characteristics and rates of successful pregnancy beyond 10 weeks within 12 months.

Primary outcome: Ongoing pregnancy at or beyond 10 weeks gestation within 12 months of an initial evaluation.

Secondary outcome: Demographic and clinical characteristics of women with uRPL including VPL, NVPL, and a mixed type of pregnancy loss.

Participant selection

Inclusion criteria

- Women 18 years old or older with a history of two or more pregnancy losses who had a complete clinical assessment (according to ASRM guidelines) by a gynecologist at BCWH, RPL center for Reproductive Endocrinology between January 1, 2011, and August 31, 2017.

Exclusion criteria

- Women with a history of:
    a) Only one pregnancy loss at < 10 weeks gestation
    b) Any pregnancy beyond 20 weeks gestation.

1.9.2 Study 2: Developing a prognostic classification of unexplained recurrent pregnancy loss.

Objective

To examine demographic and clinical characteristics in women with Type 1 and Type 2 uRPL and the association with a successful pregnancy (an ongoing pregnancy at or beyond 10 weeks gestation) within 12 months after the visit at the RPL clinic between January 1, 2011, and August 21, 2017.

Hypothesis

- We hypothesized that women with Type 2 uRPL would have a higher proportion of successful pregnancies (beyond 10 weeks gestation) as compared to women with Type 1 uRPL.

Primary outcome: Presence of a successful pregnancy at or beyond 10 weeks' gestation within 12 months after the initial evaluation at the RPL clinic.

Secondary outcome: Demographic and clinical characteristics in women with Type 1 and Type 2 uRPL.

Participant selection

Inclusion criteria:

- Women with unexplained recurrent pregnancy losses categorized as Type 1 or Type 2 uRPL, who were 18 years of age or older and who had a complete clinical assessment (according to ASRM guidelines) by a gynecologist at BCWH, RPL center for Reproductive Endocrinology between January 1, 2011, and August 31, 2017.

Definitions:

- Type 1: women with uRPL who were ≥ 35 years old at the time of the first visit (at RPL clinic), and had a history of only non-visualized pregnancy losses (NVPL).
- Type 2: women with uRPL who were < 35 years old at the time of the first visit (at RPL clinic) and had a history of NVPLs or both (NVPL or VPL, i.e., mixed type).

- Outcome: The first pregnancy within 12 months after the initial evaluation (the possible outcomes: no pregnancy, pregnancy at or beyond 10 weeks, and miscarriage at < 10 weeks gestation).

Exclusion criteria

- Women with a history of:
    a) Only one pregnancy loss at < 10 weeks gestation
    b) Any pregnancy beyond 20 weeks gestation.

Chapter 2: Types of previous miscarriage and rates of successful pregnancy in women with uRPL: a retrospective cohort study.

2.1 Introduction

Reproduction in humans is considered a very inefficient process and one of its complications is miscarriage. The probability of a successful conception during each menstrual cycle is about 30% and among these, 50% result in miscarriage. Most (approximately 75% of all miscarriages) of these miscarriages do not exceed 12 weeks of gestation and are therefore termed as early pregnancy losses or first trimester losses [24,32,153]. Very early pregnancy losses are not clinically recognized as most of them occur prior to implantation [153]. The rate of pregnancy loss falls to 15% - 20% after the implantation has occurred or that period is crossed [154]. Among them, about 50%-60% of the first trimester losses are found to be due to chromosomal abnormalities [155-158].

Genetic analysis of the products of conception (POC) collected during uterine evacuation or terminations could be very helpful [18-21]. Various reasons to examine POC for the pregnancy losses have been proposed by different studies [18-21]:

- To evaluate the morphology of the gestational sac i.e. the chorionic villi, decidua, endometrial vessels, glands, and the implantation site of the pregnancy.
- To rule out the possible causes resulting in recurrent pregnancy losses.
- To evaluate the presence of any dysmorphic phenotype and the development of an embryo or early fetus.
- To recognize the necessity for more genetic studies to be conducted, if needed.

Pathological and cytogenetic evaluation of the POC's is thus should be considered as an important and reliable source of information.

2.2 Terminologies

Early pregnancy loss is a term that covers different clinical scenarios. To deal with this complication it is very important to know the difference between all types of early pregnancy loss for a better prognosis, treatment, and follow-up of the patients [2,159].

Early pregnancy loss includes four different clinical states:

- When the transvaginal ultrasound (TVS) or the histological findings confirm an intrauterine pregnancy demise before 12 weeks' gestation, it is termed as a miscarriage;
- Ectopic pregnancy (EP) is a pregnancy shown by laparoscopy or TVS to be outside of the uterine cavity;
- A biochemical pregnancy loss is a pregnancy loss after a positive pregnancy test without TVS being performed
- In the scenario where the pregnancy test was positive and, an ultrasound was performed but no intrauterine pregnancy or ectopic pregnancy was identified as pregnancy of unknown location (PUL) [2] .

PUL has four possible outcomes: an ongoing intrauterine pregnancy, an ectopic pregnancy, an intrauterine miscarriage, or a failed PUL. About 50%-70% are failed PUL as the true location of the pregnancy loss is not usually known because these pregnancy losses are not clinically visualized [160-163]. Considering all the terminologies used under early pregnancy loss, biochemical pregnancy loss, and PUL share the same prognostic importance and are therefore grouped as 'non-

visualized pregnancy losses' (NVPL). A NVPL is defined as a pregnancy loss that is confirmed earlier by a positive serum beta-hCG but is not visualized if TVS is performed [164].

NVPL's are encountered among the RPL population partly because ultrasound testing during the very early pregnancy period may not be easily available [165]. ASRM Practice committee definition does not recommend the inclusion of NVPL's in the definition of RPL as they are not clinically recognized and thus do not qualify for the evaluation [166]. To include NVPLs in the definition criteria of RPL is controversial and has been debated for years. One way to find out if NVPLs are clinically relevant to be included in the definition and evaluation criteria for RPL is by investigating the incidence of NVPLs among the total RPL population and if they have any negative effect on subsequent live births.

NVPL in uRPL: Most of the pregnancy losses remain unexplained and, knowing the prevalence of NVPLs and incidence of subsequent live births could be one of the steps towards a positive outcome for the patients as well as for their caregivers. Data about the adverse pregnancy outcome or the incidence of ongoing pregnancies in the uRPL group of women are sparse. A retrospective study done at our BCWH RPL clinic in the cohort of 1060 RPL women, found that NVPL were frequently encountered with a prevalence of 14% in this cohort. The ESHRE criteria were followed to define the inclusion and exclusion criteria for the diagnosis of RPL in this cohort of women. This study is yet to be published.

In another retrospective study, Kolte et al., 2014, found that women with NVPLs had the same prognosis as those with clinically recognized pregnancy losses (visualized pregnancy loss) and they recommended including NVPLs in the definition [164]. They included women with three or more consecutive pregnancy losses before 12 weeks' gestation in the group of uRPL women. Kolte

et al., 2014, were the first to study this and their results supported the inclusion of NVPLs in the definition of RPL [164].

Correct implantation and placentation are critical for a successful pregnancy. In women with RPL, abnormal signaling occurring during the time of preimplantation that may create an unreceptive environment during the early phases of pregnancy. Knowing that 50% of the RPLs are unexplained, this raises a question of considering and evaluating the neglected number of NVPLs. Considering all the facts, we focused on the women with a history of unexplained recurrent pregnancy loss and hypothesized that NVPL and VPL have prognostic value for determining the probability of a future successful pregnancy defined as an ongoing pregnancy at ≥ 10 weeks' gestation.

2.3 Materials and methods

In this retrospective cohort study, I used a database of women referred to British Columbia's Women's Hospital (BC Women's Hospital: BCWH), RPL Clinic (a tertiary referral center), Vancouver, British Columbia, Canada from January 1, 2011, to August 31, 2017. The study began after the approval by the University of British Columbia Research Ethics Board (Approval number: H13- 03306). Data on 1311 women with a history of RPL was extracted from the Research Electronic Data Capture (REDCap) data management platform at BC Children's Research Institute (BCCHRI) [167]. Their clinical evaluation was done following the American Society of Reproductive Medicine guidelines (ASRM) [5].

Clinical history

At the first visit after referral to our RPL clinic, patients underwent a complete clinical assessment and physical examination done by a gynecologist. With the help and guidance provided by the gynecologists at the BCWH RPL clinic, we created a very detailed and user-friendly RPL patient intake form (appendix A). The form included questions related to the patient's personal, educational and occupational background, gynecological history, medical and family history of the patient, and their previous or current partners. There were brief questions about pregnancy history comprised of the number of pregnancy losses, term deliveries (at or more than 37 weeks), preterm deliveries (between 20 and 36+6 weeks), miscarriages at < 20 weeks, pregnancies of unknown location, tubal pregnancies and about the number of living children.

For the analysis, the patient's age was subdivided into subgroups: 19-29, 30-34, 35-39, and ≥40 years. Other variables considered for the analysis from the history were the number of pregnancy losses (2, 3, ≥ 4), primary or secondary pregnancy loss, history of infertility (primary

or secondary), therapeutic abortions, and ectopic pregnancies. Primary RPL indicates multiple losses in women who have had no live births in the past and secondary RPL refers to multiple losses in a woman with at least one prior live birth or pregnancy beyond 20 gestational weeks [36,168].

Physical examination

A full general examination, including blood pressure (BP), pulse rate, height, and weight, were done first. Further, the examination proceeded with the pelvic and bimanual examination. For the analysis, BMI was calculated and recorded (kg/m^2) and was further subdivided into categories: underweight (<18.5), normal BMI (18.5-24.9), overweight (25-29.9) and obese (≥30).

RPL investigations

At our clinic, a full workup is ordered at the initial visit to identify the treatable causes. ASRM guidelines recommend genetic karyotypes of both the partners, evaluation of the uterine cavity (sonography, hysteroscopy, or hysterosalpingography), and immunologic tests for detecting antiphospholipid antibody syndrome (lupus anticoagulant, anticardiolipin antibodies, β2 glycoprotein), hormone assays and other metabolic factors along with the lifestyle variables of both the partners, i.e., smoking, use of alcohol, stress; and the further following information about the previous miscarriages or successful pregnancies [5,34,169] (Appendix B).

Genetic testing

Products of conception

Genetic analysis of the products of conception of the RPL patients is not recommended for routine investigations but it could be performed for informative purposes [33].

According to ASRM[5] and in the most recent ESHRE guidelines [33], for cytogenetic analysis of products of conception (POC), array comparative genomic hybridization (CGH) has been recommended over traditional karyotyping due to decreased maternal cell contamination [5,33]. Chromosomal microarray analysis was introduced at the BCWH RPL clinic in 2017. Previously, only conventional karyotyping was being used for the POC evaluation. Conventional karyotyping could only identify chromosomal rearrangements and copy number variants (CNVs) up to a ~5Mb resolution whereas chromosomal microarray analysis (CMA) can identify unbalanced CNVs even under 1 Mb resolution [33]. In our center, we considered analyzing the POC evaluation for the second and subsequent miscarriages. Aneuploidy in both or either of the second and third miscarriages was considered abnormal [101].

Parental karyotyping

Parental karyotypes were reviewed by a geneticist, major rearrangements (e.g., mosaics and balanced translocations) were considered abnormal. Abnormal result in karyotyping in either of the partners or both the partners was considered as abnormal.

Endocrine investigations

Diabetes, thyroid dysfunction and hyperprolactinemia are some of the endocrinological disorders known to be associated with RPL. Following the clinical guidelines, all the patients had assays for TSH, thyroid peroxidase antibodies and prolactin, fasting blood glucose levels and hemoglobin A1C (HbA1C). For this study cohort, we considered serum thyroid stimulating hormone (TSH), below 10 IU/mL as a normal cut off level for frank hypothyroidism (criteria ≥10 IU/mL abnormal, 4-10 IU/mL subclinical, 2.5-4 IU/mL borderline subclinical, and <2.5 IU/mL normal). Thyroid autoantibodies were tested only if abnormal TSH findings confirmed the presence of thyroid disease.

Anatomical investigations

Uterine cavity evaluation is done to rule out both congenital and acquired abnormalities. For the uterine anatomical evaluation, a two-dimensional ultrasound followed by hysterosalpingography or hysteroscopy was performed. Pelvic MRI is only done for the cases with complex anatomic defects [5,34].

Autoimmune investigations

To confirm the diagnosis of antiphospholipid syndrome (APS), blood tests are done to check three APS antibodies: lupus anticoagulant (PT 9.0-11.4 sec), anticardiolipin antibodies (IgG <20 GPL-u/ml, <1.3 MOM and IgM <20 MPL-u/ml, <1.3 MOM) and anti β_2 glycoprotein 1 antibodies (IgG <20 GPU and IgM <20 SMU). All values in the parentheses are normal values above which the test result is considered abnormal. Positive antiphospholipid antibodies have to be repeated in 12 weeks to confirm the diagnosis [5].

Inherited thrombophilia screening was ordered if the patient had a personal history or had a first-degree relative with a suspected high risk of thrombophilia [5,34]. For thrombophilia screening, Factor V Leiden gene mutation, antithrombin III, and prothrombin gene mutation (G20210A) are usually assessed. For this cohort study, we only considered analyzing the presence or absence of antiphospholipid antibody syndrome by testing the aPL antibodies. International consensus classification criteria for APS was followed for the evaluation (Figure 2.1) [5,170,171].

Figure 2.1 International consensus classification criteria for the antiphospholipid syndrome (APS). Adapted from [5,170,171]

International consensus classification criteria for the antiphospholipid syndrome (APS)

APS is present if one of the following clinical criteria and one of the laboratory criteria are met:

Clinical

1. Vascular thrombosis 2. Pregnancy morbidity

a. One or more unexplained deaths of morphologically normal fetuses after the 10^{th} week of gestation by ultrasound or direct examination of the fetus.

b. One or more premature births of a morphologically normal neonate before the 34^{th} week of gestation because of eclampsia or severe pre-eclampsia or recognized features of placental insufficiency.

c. Three or more unexplained consecutive spontaneous abortions before the 10^{th} week of gestation with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

Laboratory

1. Lupus anticoagulant present in plasma on two or more

   occasions at least 12 weeks apart, or

2. Anticardiolipin antibody of IgG or IgM isotype in serum or

   plasma present in medium or high titer (>40 GPL or MPL or > 99^{th} percentile), on two or more occasions at least 12 weeks apart, or

3. Anti-b2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titer greater than the 99^{th} percentile), present on two or more occasions at least 12 weeks apart.

Practice Committee. Recurrent pregnancy loss. Fertil Steril 2012.

Ongoing pregnancy monitoring

After RPL investigation and necessary treatment, most women were able to conceive and attended the BCWH RPL clinic. Ongoing pregnancy is the first pregnancy that occurred during the ongoing care at the clinic [172-174]. The pregnancy monitoring at our clinic started with the following steps (Appendix C):

- **Referral:** Referrals for pregnancy monitoring were received either by the care providers or as a self-referral by the patients.
- **Documentation:** RPL pregnancy monitoring intake form was completed by one of the registered nurses at the clinic during the initial visit of the patient.
- **Laboratory investigations:** All patients were required to have laboratory investigations done at 5 weeks of gestation and earlier in cases presenting with bleeding during pregnancy. For patients with a history of ectopic pregnancy, an ultrasonographic examination appointment was made. Laboratory investigations included: serum beta-hCG done twice (48 hours apart), blood group/Rhesus, Rubella, Varicella, RPR, HIV, hepatitis B, hepatitis C screening. TSH levels were ordered for the patients with a prior history of positive thyroid peroxidase antibodies (TPO) or those who were on levothyroxine therapy or had TSH ≥ 2.5 IU/mL. Women working in hospitals or close contact with children were advised to undergo cytomegalovirus (CMV), Toxoplasmosis, and Parvovirus serology. Finally, women on anticoagulant dalteparin therapy as a treatment had a complete blood count (CBC) to check the platelet levels first at the time of their first visit and then again after two weeks.
- **Phone calls:** Patients were called by one of the registered nurses at our clinic to review the test results and to schedule their initial pregnancy monitoring visit according to the

following criteria: Firstly, for the initial hCG value < 1500 IU/L, the predicted two-day minimal rise is around 49%, for the values between 1500 – 3000 IU/L the predicted rise is about 40% and for values >3000 IU/L it is 33%. In case of an appropriate rise in beta hCG levels, the initial ultrasound visit with the clinician was scheduled at 6 to 7 weeks of gestation. In case of an inappropriate rise in beta hCG, the initial ultrasound visit along with the clinician visit was scheduled at 5 to 6 weeks of gestation. Patients were called in to review the reports with the clinician in case of decreased level of hCG was reported. Secondly, during pregnancy, fetal iso-immunization can occur if the mother is Rh-negative and the fetus is Rh-positive (Rh incompatibility). For the women who were Rh-negative, Anti-D immunoglobulin was advised as treatment if there was bleeding during the pregnancy. Thirdly, immune status of the patients was reviewed precisely especially for the CMV, Toxoplasmosis, and Parvovirus serology results

- **TSH:** Consultation with the RPL clinician regarding the start of levothyroxine treatment was initiated for the patients who had TSH ≥ 2.5 IU/mL.
- **History of hypothyroidism:** Patients with a history of hypothyroidism and who had TSH > 2.5 IU/mL were advised to double the dosage of levothyroxine for two days per week to account for the 30% increase in demand during pregnancy.
- **Ultrasound:** Ultrasonographic (USG) examination was initiated along with the clinician visit for all RPL women at 6 to 7 weeks of gestation. Women with a history of ectopic pregnancy or those who had an abnormal rise in beta-hCG were scheduled for USG and a clinician visit at 5 to 6 weeks' gestation.
- **Folic acid supplementation:** Folate (vitamin B9) is an essential nutrient required for DNA replication and certain enzymatic reactions involved in amino synthesis and vitamin

metabolism. There is an increase in demand for folate during pregnancy as it is required for the growth and development of the fetus. Deficiency of folate has been known to be associated with abnormalities like anemia and peripheral neuropathy in mothers and congenital abnormalities in fetuses including neural tube defects [175,176]. Therefore, all the patients were reminded to take folic acid supplementation of 400μg daily [177].

- **Internal medicine consultation:** Patients who were on low molecular weight heparin (LMWH): dalteparin, were advised to have their platelets level checked at the initial visit and the next two weeks later. Further going ahead, an appointment was booked for them with an Internal Medicine clinician at 10 weeks of gestation to rule out any developing complications from using LMWH.
- **Embryopathology:** This is an important step toward finding the underlying cause in cases of recurrent pregnancy losses. Patients were advised to collect the tissue of the products of conception and to refrigerate in a plastic container that was to be submitted to the Women's Health Center in the event of miscarriage.
- **Follow-up ultrasound:** For those with an ongoing pregnancy, the final follow-up ultrasound along with the clinician visits was scheduled at 9 to 10 weeks of gestation.
- **Discharge:** The patients were discharged from the RPL pregnancy monitoring program at the time of the last follow-up ultrasound conducted 9 to 10 weeks of gestation or earlier in the event of confirmed fetal demise. Further, referral to a prenatal care provider was given if needed.

Successful pregnancy was defined as an ongoing pregnancy lasting 10 weeks' gestation or longer. Three possible outcomes were: 1) successful pregnancy; 2) pregnancy ending in a miscarriage at < 10 weeks' gestation, and 3) no pregnancy.

Pregnancy characteristics

Medical history was obtained from patients referred to the RPL clinic regarding the previous pregnancies and their outcomes from the patient records and/or by self-report. For those with a previous successful pregnancy, the information included the date of the last menstrual period, information about any kind of fertility treatment used for that pregnancy and outcome of the pregnancy. Gestational age was calculated from the last menstrual period and by the ultrasonographic examination in weeks and days. Details of the pregnancy during the first trimester was recorded as (a) Non-visualized pregnancy: biochemical pregnancy loss or resolved pregnancy of unknown location (PUL) or treated PUL, (b) Miscarriage: early or anembryonic or with yolk sac or embryonic, and (c) Ectopic pregnancy or (d) an ongoing pregnancy beyond 10 weeks gestation.

Patient characteristics

Details for 1311 women were included in the database (Figure 2.1). Of these, 58 women with only one prior fetal demise after 20 weeks of gestation were excluded as they did not meet the criteria of RPL. Evaluation of the couples following a single miscarriage is not recommended[169]. A total of 1253 women with RPL were eligible and evaluated following the ASRM guidelines. Among these cases, we had data from 799 couples who underwent maternal and paternal karyotyping, 1080 women underwent autoimmune testing, 713 women had a uterine

evaluation done and TSH results of 1176 women were available. The remaining women had either evaluation results missing or their data was incomplete in the database. Therefore, in the cohort of 1253 cases, maternal and paternal karyotypes that were found to be normal were 755 (60.2%) and were abnormal in 44 (3.5%), and autoimmune antibodies were negative in 998 (79.7%) and positive in 82 (6.5%), uterine anatomy was normal in 557(44.4%) and abnormal in 156 (12.4%) and TSH was normal in 1171 (93.5%) and abnormal in 5 (0.4%).

Patients with any of the above abnormal testing results were considered to have explained recurrent pregnancy losses (n= 265, 21.1%), and all those having all normal test results or normal or missing test results were categorized as having unexplained recurrent pregnancy losses (n=972, 77.6%). 16 women (1.3%) had all test results missing or unknown and were excluded from the study.

Table 2.1 Participant selection flow chart.

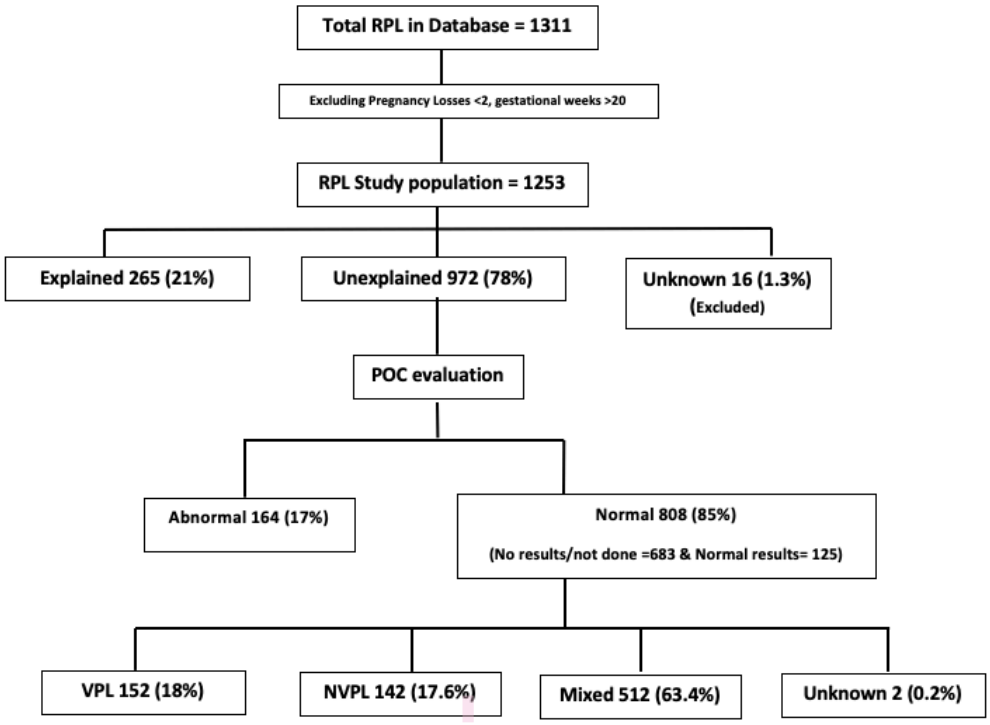
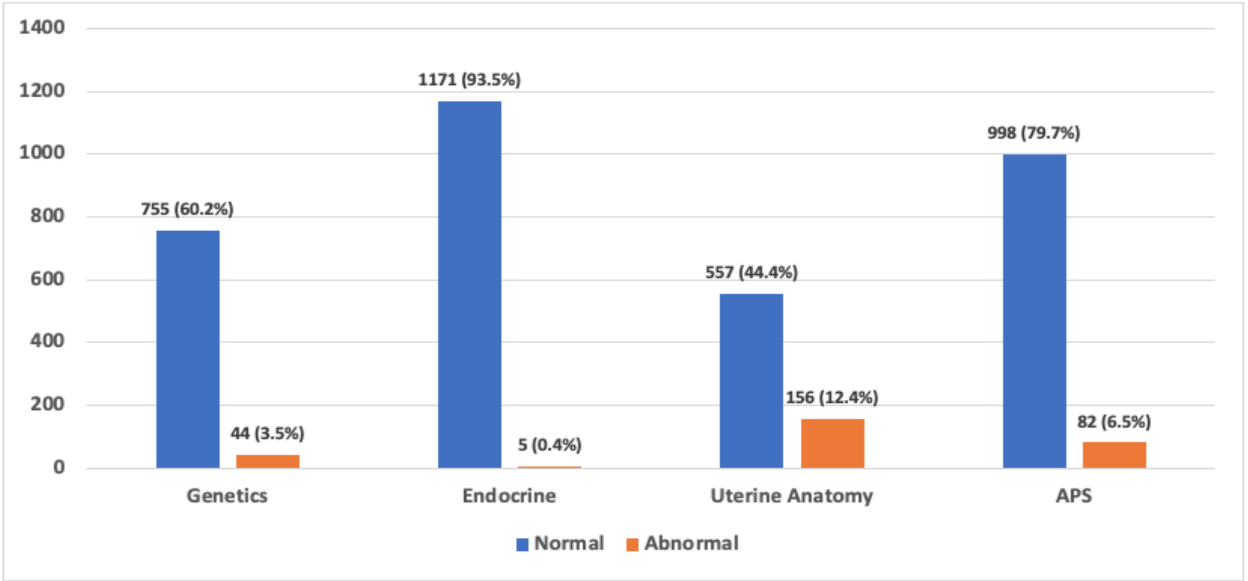


Figure 2.2 Determination of normal and abnormal testing in the RPL study population.



The unexplained recurrent pregnancy loss group (uRPL) was further evaluated based on the cytogenetic testing performed on the products of conception (POC) from the previous second and third miscarriages prior to the ongoing pregnancy that occurred at our RPL clinic. POC cytogenetics was not performed on the cases with the history of normal parental karyotypes. In our cohort, a total of 972 patients, 164 (16.8%) were found to have abnormal POCs and 808 (83.1%) were normal in previous miscarriages. In 15% (n=125) of the cases, POC cytogenetics was not performed and 84.5% (n=683) tested negative for any abnormality and were considered normal. Therefore, we can stipulate that only 808 patients had 'truly' unexplained recurrent pregnancy losses. We further restricted our study to these 808 patients and examined the prevalence of VPL, NVPL, and the combination of both types of pregnancy losses.

Data analysis

Data were analyzed using SPSS version 26.0. The study consisted of two-level analyses. In the first level of the analysis, descriptive statistics were evaluated for the clinical characteristics. In the second-level analysis, a bivariate analysis was performed to compare women with different types of uRPL with respect to demographic and clinical characteristics using a chi-square test. A *p-value* of less than 0.05 was used to determine the significance of statistical tests.

Key variables/ measurements

We compared three groups of patients based on the types of their recurrent pregnancy loss: VPL (visualized pregnancy loss), NVPL (non-visualized pregnancy loss), and Mixed (a combination of both). We went through the history of each patient with confirmed uRPL and

characterized each using the terminology provided by the European Society for Human Reproduction and Embryology (ESHRE) guidelines [1,164,178].

The demographic and clinical characteristics of interest included age (19-29, 30-34, 35-39 and ≥40 years) at the time of the last pregnancy loss (before the successful pregnancy), BMI category: underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), obese (≥30 kg/m^2), number of prior pregnancy losses (2, 3, ≥ 4), primary or secondary pregnancy loss, history of infertility (no, primary or secondary), history of therapeutic abortions (yes/no), and history of ectopic pregnancies (yes/no). Primary RPL indicates multiple losses in a woman who has had no prior live births, whereas secondary RPL refers to multiple losses in a woman with at least one prior live birth or a pregnancy beyond 20 gestational weeks [36,168]. All the information was extracted from the REDCap database that was used to store the patient data.

2.4 Results

The main objective was to evaluate the demographic, laboratory, and clinical factors in women with uRPL with respect to different types of pregnancy losses (only VPL, only NVPL, and mixed). Further, we compared the rates of successful pregnancies (ongoing pregnancy at or beyond 10 weeks' gestation) in these three groups after a follow up within 12 months from the initial visit at the RPL clinic.

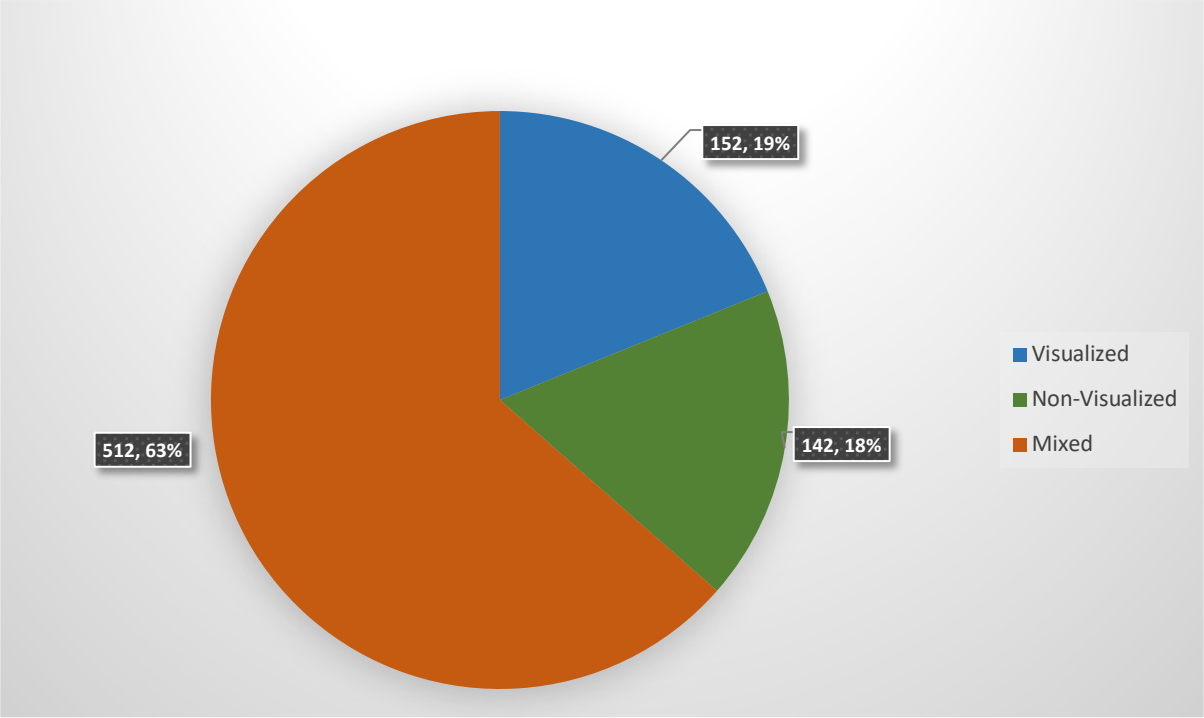
2.4.1 **Descriptive statistics**

2.4.1.1 Demographic characteristics of women with uRPL

The total number of women with 'true' uRPL was 808. The prevalence of patients with only NVPL was 18% (n=142), while 19% (152) of patients had only VPLs. The remaining 63%

(512) uRPL women were found to have a mixture of both (mixed) (Figure 2.3). Two patients had insufficient information to be categorized under one of the types and therefore were excluded. The total number of patients included in the analyses was 806.

Figure 2.3 Prevalence of VPL, NVPL and combination of both in pure uRPL group.



The prevalence of primary pregnancy loss was higher than secondary pregnancy loss among women with uRPL (59.6% vs 40.4%). A total of 43 women had an ectopic pregnancy and 180 had a history of therapeutic abortions.

About 70% of women were 30 to 39 years of age. The mean age of patients in the study was 34.52 (SD ± 6.18). The descriptive characteristics of all 808 patients are presented in Table 2.2**.** The age distribution of women with all types of pregnancy losses (VPL, NVPL, and Mixed) are presented in Table 2.3.

Table 2.2 Percentage distribution of clinical characteristics of uRPL patients.

Variables		Categories	Frequencies	Percentages
Age		19-29 years	89	11.2
		30-34 years	274	34.5
		35-39 years	284	35.8
		≥40 years	147	18.5
Mean		34.52		
Standard deviation		6.18		
Total			794	100
BMI (kg/m^2)		Underweight (<18.5)	16	5
		Normal (18.5-24.9)	168	52.5
		Overweight (25-29.9)	75	23.4
		Obese (≥30)	61	19.1
Mean		26.33		
Standard deviation		15.72		
Total			320	100
Pregnancy loss		Primary	480	59.6
		Secondary	320	40.4
Total			806	100
History of infertility		No	583	81
		Primary	66	9.2
		Secondary	71	9.8
Total			720	100
Therapeutic abortions		No	626	77.7
		Yes	180	22.3
Total			806	100
Ectopic pregnancy		No	763	94.7
		Yes	43	5.3
Total			806	100
Caucasian		No	212	26.3
		Yes	216	26.8
Total			806	100
Spontaneous abortions		2 to 3	600	74.4
		> 3	206	25.6
Total			806	100
Types of pregnancy loss		Visualized	152	18.9
		Non-Visualized	142	17.6
		Mixed	512	63.5
Total			806	100
Follow-up outcomes	**Miscarriage**	< 10 weeks	282	35
	Ongoing pregnancy	≥ 10 weeks	397	49.2
	No pregnancy		127	16
Total			806	100

*Missing values for Age = 12, BMI = 480 and history of infertility = 86

Table 2.3 Types of pregnancy losses and age distribution.

Age groups	VPL	NVPL	MIXED
19-29	16 (10%)	17 (12%)	56 (11%)
30-34	59 (39%)	52 (38%)	163 (32%)
35-39	56 (37%)	49 (35%)	179 (35%)
≥40	21 (14%)	20 (14%)	106 (21%)

2.4.1.2 Clinical characteristics of women with uRPLs

The proportion of women with ≥ 4 prior losses in the mixed group (i.e., a combination of clinical and non-visualized pregnancy losses) was 34.2% as compared with only 14.0% and 7.2% in women with only non-visualized and only visualized pregnancy losses, respectively. 28% (145/512) of uRPL women with mixed type of pregnancy losses had a history of therapeutic abortions as compared to the NVPL 18.3% (26/142) and VPL 6% (9/152). Primary pregnancy losses were in the VPL group 70% (106/152) as compared to the NVPL group 53.5% (76/142) and mixed pregnancy loss groups 58.2% (298/512). Descriptive characteristics are shown in Table 2.4.

Table 2.4 Basic clinical characteristics of women with uRPLs.

Variables	VPL N=152	NVPL N=142	MIXED N=512
No. of pregnancy losses			
2 to 3	141 (93.0%)	122 (86%)	337 (66%)
≥ 4	11 (7.2%)	20 (14.0%)	175 (34.2%)
Total	152	142	512
Age			
19-29	16 (10.5%)	17 (12.3%)	56 (11.1%)
30-34	59 (39.0%)	52 (37.7%)	163 (32.3%)
35-39	56 (37.0%)	49 (35.5%)	179 (36%)
≥ 40	21 (14.0%)	20 (14.5%)	106 (21.0%)
Total	152	138	504
Missing	0	4	8
Prior therapeutic abortion			
Yes	9 (6.0%)	26 (18.3%)	145 (28.3%)
No	143 (94%)	116 (82%)	367 (72%)
Total	152	142	512
Prior ectopic pregnancy			
Yes	5 (3.2%)	12 (8.4%)	26 (5%)
No	147 (96.7%)	130 (91.5%)	480 (95%)
Total	152	142	506
Missing	0	0	6
History of infertility			
No	111 (79%)	98 (84%)	374 (81%)
Primary	16 (11.4%)	5 (4.4%)	45 (10%)
Secondary	13 (9.3%)	14 (12%)	44 (9.5%)
Total	140	117	463
Missing	12	25	49
BMI			
Underweight (<18.5)	3 (4.5%)	2 (3.2%)	11 (5%)
Normal (18.5-24.9)	31 (47%)	38 (61.3%)	99 (44%)
Overweight (25-29.9)	20 (30.3%)	15 (24.2%)	40 (18%)
Obese (≥ 30)	12 (18.1%)	7 (11.3%)	75 (33.3%)
Total	66	62	225
Missing	86	80	287
Pregnancy loss			
Primary	106 (70%)	76 (53.5%)	298 (58.2%)
Secondary	46 (30.2%)	66 (46.4%)	214 (42%)
Total	152	142	512

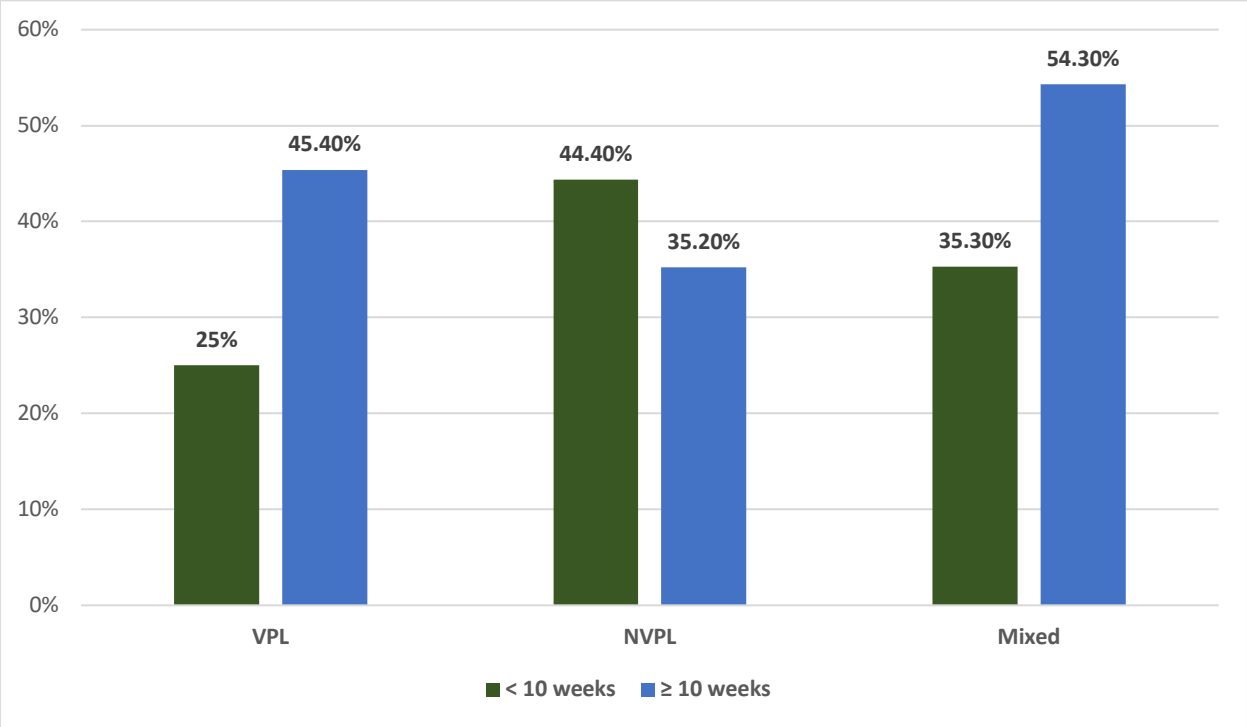
2.4.1.3 Ongoing pregnancy at 10 weeks gestation and beyond

Among the cohort of 806 uRPL women, 397 (49.2%) had pregnancy reaching 10 weeks' gestation or more, and 282 (35%) pregnancies were miscarried. 127 (15.8%) uRPL women did not have pregnancies within 12 months after the first visit. There was a higher number of successful pregnancies reaching 10 weeks or more among the Mixed type of pregnancy loss group: 54.3% (278/512) in comparison to the VPL and NVPL groups with 45.4% (69/152) and 35.2% (50/142) respectively. Comparing VPL and NVPL with respect to the incidence of miscarriages, a lower proportion of miscarriages occurred among the VPL group (25.0%; 38/152) than in the NVPL group (44.4%; 63/142); (Table 2.5 and Figure 2.5).

Table 2.5 Outcomes (subsequent pregnancy) in women with three types of pregnancy loss.

Outcome	VPL N (%)	NVPL N (%)	Mixed N (%)	Total N (%)
Miscarriage at < 10 weeks	38 (25.0)	63 (44.4)	181 (35.3)	282 (35)
Ongoing pregnancy at ≥ 10 weeks	69 (45.4)	50 (35.2)	278 (54.3)	397 (49.2)
No pregnancy	45 (29.6%)	29 (20.4%)	53 (10.3%)	127 (15.8)
Total	152	142	512	806

Figure 2.4 Outcome of the first pregnancy at the RPL clinic among different types of pregnancy losses.



2.4.2 Binary logistic regression

We performed binary logistic regression to assess independent associations between demographic and clinical characteristics and the type of uRPL. The mixed category of pregnancy losses was excluded from the analysis, because it is a combination of both VPL and NVPL, and the result would be expected to fall in between these two extremes (only VPL and only NVPL). We did not include BMI in the regression model because of the large number of missing values. Adjusted odds ratio (AOR) was used to describe the independent associations between women's characteristics and their VPL's versus NVPL history. 95% confidence intervals (CI) were used to assess the precision of these associations. Overall, the model fit the data well, correctly classifying two thirds of cases in each category, visualized pregnancy losses (65%), and non-visualized pregnancy losses (69%). The model explained 20% (Nagelkerke R^2) of the variance. The second

regression analysis examined the independent association between the type of uPRL (VPL vs NVPL) and the outcomes of subsequent pregnancy among women who become pregnant.

Of the 6 characteristics, 3 were found to be statistically significantly different between women with VPL and NVPL: a history of primary or secondary pregnancy losses (AOR = 1.961, 95% CI = 1.192 – 3.225), history of primary infertility (AOR = 0.318 and 95% CI = 0.109 – 0.926), and history of therapeutic abortions (AOR 3.274 and 95% CI = 1.425 – 7.5233) (as shown in Table 2.6). Primary pregnancy loss was significantly less common among NVPL as compared to the VPL. uRPL women with NVPLs were more likely to have a history of primary infertility and had undergone therapeutic abortions in the past as compared to the women with VPLs.

Women who had VPL were significantly more likely to have successful pregnancy beyond 10 weeks of gestation; AOR = 2.427, 95% CI = 1.335 – 4.424 (Table 2.7). In other words, among women who became pregnant within 12 months after their first visit to the RPL clinic, those who had VPL were less likely to have a miscarriage as compared with women who had NVPL. These results were adjusted for other variables presented in Table 2.6.

Table 2.6: Logistic regression results expressing independent associations between demographic and clinical characteristics among women with VPL vs NVPL (reference category).

Variables	Subcategories	*p*-value	AOR (95% CI)
Age	< 35	0.495	0.848 (0.529 - 1.361)
	≥ 35		
Number of pregnancy losses	> 3	0.165	1.789 (0.787 - 4.064)
	2 to 3		
Pregnancy loss	Primary	**0.008**	1.961 (1.192 - 3.225)
	Secondary		
History of infertility			
Primary	No	**0.036**	0.318 (0.109 - 0.926)
	Yes		
Secondary	No	**0.686**	0.836 (0.351 - 1.990)
	Yes		
Therapeutic abortion	No	**0.005**	3.274 (1.425 - 7.523)
	Yes		
Ectopic pregnancy	No	0.099	2.593 (0.835 - 8.057)
	Yes		

*Adjusted for all other variables in this table

Table 2.7 Logistic regression results expressing the associations between VPL and successful pregnancy, among women with unexplained recurrent pregnancy loss who became pregnant within 12 months after the initial visit at the RPL clinic.

Types of unexplained recurrent pregnancy loss	Length in weeks	*p* value	AOR* (95% CI)
VPL	**< 10**	**0.044**	**2.427 (1.335 - 4.424)**
NVPL	**≥ 10**		**RC**

*Adjusted for variables in Table 2.6, RC: reference category

2.5 Discussion

In this study of unexplained recurrent pregnancy loss among patients referred to our tertiary referral center BCWH, RPL Clinic, we performed regression analysis to compare the demographic and clinical factors among women with VPL and NVPL. A successful pregnancy (reaching 10 or more weeks) was less likely among women with NVPL as compared to women with VPL in a cohort of patients with uRPL.

The descriptive analysis was done including all three groups of women with uRPL, i.e., VPL, NVPL, and a mixed type. Overall 54.3% of the women were 35 years old or older. Among women with VPL, 45.4% had a successful pregnancy (> 10 weeks) and 25.0% had a miscarriage, among those with NVPL had 35.2% had a successful pregnancy (>10 weeks) and 45.4% had a miscarriage.

We did not find any significant differences in age, a number of pregnancy losses, and ectopic pregnancies, among the NVPL group in comparison to the VPL group. In regard to the history of ectopic pregnancy, there were no significant differences among these groups. This result is in contrast with the results provided by one of the studies by Kolte et. al, 2015 [164]. They documented that the history of well treated ectopic pregnancy with surgery was significantly higher in NVPLs as compared with women with VPLs.

In our cohort of women with uRPL, 18% had exclusively non-visualized pregnancy losses, while 82% had either VPL or both types. The majority of women in our study had two to three prior pregnancy losses. Furthermore, we compared the clinical characteristics of the uRPL women among all three types of pregnancy losses (VPL, NVPL, Mixed). We have also observed that a larger proportion of women with uRPLs had primary pregnancy losses especially in women with VPL. In our cohort, the majority of uRPL women with NVPLs were found to have a history of

therapeutic abortions, and one of the reasons for the need for a therapeutic abortion before 10 weeks could be when the tests show that the pregnancy would result in a child with severe birth defects.

We also wanted to see the percentage of successful pregnancies occurring in our cohort after undergoing treatment at the BCWH RPL clinic. Out of 806 women with uRPL, 679 pregnancies were reported. 58.5% of them had successful pregnancies (at or beyond 10 weeks' gestation) and 41.5% resulted in miscarriages. The overall percentage of successful pregnancies among the NVPL group of women was 35.2% and 45.4% among the VPL group of women. This association was statistically significant after adjustment for other demographic and clinical factors. This suggests that preclinical losses do have a prognostic value towards achieving a successful pregnancy reaching more than 10 weeks gestation and may be considered under definition and evaluation criteria of miscarriage.

Some of the previous studies reported the prevalence of NVPLs (biochemical pregnancy losses and failed pregnancy of unknown location) and VPL among the RPL group of women [179]. Others documented only biochemical pregnancy loss and failed PUL but there is a paucity of studies focusing on non-visualized pregnancies (BPL or failed PULs) in patients with RPL [179]. Kolte et al., 2014, were the first to show the effect of NVPLs (BPL combined with failed PUL), in a retrospective cohort study of uRPL patients. In their cohort of 2454 pregnancy losses, there were 1426 (58%) miscarriages reported before 12 weeks' gestation. 578 (23%) of them were biochemical pregnancy losses and 334 (16%) were failed PULs. In total, 912 (37%) were NVPLs. For the biochemical losses, the mean gestational age was 6.08 weeks and 6.59 weeks for the failed PULs. The mean gestational age reported for the clinical miscarriages was considerably higher (8.87 weeks) than the non-visualized pregnancy losses by 2.60 weeks. Among 499 women, they

found that the relative risk of having live births for each non-visualized pregnancy loss was 0.90, and for the clinical miscarriages was 0.87. There was no statistical significance found in the difference of RRs for live births accounted for by each clinical miscarriage and NVPL. Their study did not compare other clinical characteristics of the uRPL women that could prove to be a contributing factor towards the etiology of uRPL. Their study also supported the idea of inclusion of NVPLs in the definition of RPL.

Another study (Bringham et al., 1999), predicted that the overall success rate (pregnancy going beyond 24 weeks) was 75% [180]. Similarly, Lund et al. reported that in their cohort of RPL women, the incidence of at least one live birth in the five years after the first consultation was 66.7% [181]. Both the studies thus suggested that the number of clinical miscarriages before a referral is prognostically important in women with a history of recurrent pregnancy loss. The differences in the outcomes in these two studies were due to the differences in the RPL definition. RPL defined as more than three prior losses were used as the baseline inclusion criteria, but 24% of the women in Bringham et al's study had only two miscarriages [180,181]. Other studies, e.g., Bellver et. al., 2007, and Simon et. al, 1999, reported an increased number of biochemical losses using the decline in beta-hCGs level criterion specifically among women with ART pregnancies [182,183].

Our study has several limitations. First, medical history was based on recall and may not be accurate and subject to recall-bias. Second, the characteristic of women in our cohort reflects only those who attended the BCWH RPL clinic. Even though this clinic is the only tertiary center in the province of BC, it may not be representative of all women with RPL. Some women may be not able to attend the clinic for cultural or other barriers (e.g., geographic barriers to access). This has implications for the generalizability of our results. Third, we were able to observe the ongoing pregnancies for a maximum of 10 weeks, thus the ultimate pregnancy outcome (miscarriage after

10 weeks' gestation, stillbirth or, live birth) is not known. Longer follow-up is needed to ascertain the ultimate pregnancy outcomes in our cohort. This can be accomplished by linking our database with the perinatal services BC database.

Our study is one of the first to report the results of a large cohort including women with confirmed unexplained recurrent pregnancy loss. Our results underline the clinical significance of including non-visualized pregnancy losses in the definition of miscarriage, and further show differences in the rates of successful pregnancies (at or beyond 10 weeks' gestation) depending on the type of pregnancy loss in this population. Our study also suggests that exclusive NVPLs are prevalent in women with uRPL. Considering NVPLs in the definition of RPL should initiate early evaluation and treatment options for women who have been neglected in the treatment process. We also demonstrated that the majority of the patients were of mixed type, i.e. combination of visualized and non-visualized uRPLs. Our cohort included only women with confirmed uRPL and therefore our results cannot be applied to a different group of patients, for example, women whose pregnancy loss was due to chromosomal anomalies.

Chapter 3: Types of unexplained recurrent pregnancy loss group of women.

3.1 Introduction

Most of the clinical guidelines clearly state that only clinical pregnancy losses should be included in the evaluation and investigation of RPL [5,33]. The reason for not including biochemical losses is because they have a higher incidence among the general population. Nearly 60%-80% of pregnancies are assumed to end in the early preclinical phase [184,185]. Maternal age, number of pregnancy losses, and types of pregnancy losses are important factors with respect to prognosis. Maternal age is one of the most significant factors related to pregnancy loss in women with RPL[186,187] as losses often occur due to embryonic and oocyte aneuploidy [188].

About 50% of reproductive age women encounter unexplained pregnancy loss [105,189]. It has been challenging and frustrating for the clinicians to identify all underlying causes [187]. It is known that women with uRPL who are not receiving any treatment still have a good prognosis with respect to a subsequent live birth (up to 75%) [190]. The reason behind the good prognosis is because large numbers of these women are generally healthy without any underlying disease, and some repetitive pregnancy losses may occur by chance. Also, providing tender loving care (TLC) is assumed to be one of the factors associated with good prognosis [107].

Using the incidence of sporadic miscarriage in various age groups of the general population, Saravelos and Li estimated the incidence of patients suffering from recurrent miscarriage due to chance alone (Table 3.1) [107]. They concluded that the incidence of recurrent miscarriage (RM) differed with respect to the age of the women. In comparison with the younger women (in their 20's), a higher number of older women (in their 40's) were found to experience RM due to chance alone.

Table 3.1 Incidence of SM or RM occurring by chance and of RM in total, in women of different age groups[107].

Age group (years)	Sporadic miscarriage %	RM occurring by chance % (CI)	RM occurring in total (%)
20-24	11	0.13 (0.129 – 0.131)	-
25-29	12	0.17 (0.169 – 0.171)	0.4
30-34	15	0.34 (0.338 – 0.342)	~ 1
35-39	25	1.56 (1.557 – 1.564)	~ 3
40-44	51	13.3 (13.29 – 13.31)	-

Apart from chance, some women have miscarriages due to unidentified pathology. It is assumed that one out of three women might have been exposed to environmental risk factors or have unidentified endogenous pathologies. Other factors suggested by prior studies include smoking, alcohol, obesity, occupational hazard exposure, immunological pathology due to an increased number of uterine natural killer cells, and endocrinological and endometrial abnormalities including impaired decidualization [113,191-196]. The treatment options for these conditions remain unproven.

3.1.1 **Type 1 and Type 2 uRPL**

Saravelos and Li suggested creating two distinct categories of women with uRPL, Type 1, and Type 2. The following clinical factors were used to define these groups:

- Age: Saravelos and Li in their study found that in comparison with younger women (20-24 years), older women (40-44 years) were one hundred times more likely to have suffered from RM due to chance alone [107].

- Type of pregnancy loss (clinical vs biochemical): As mentioned earlier, the incidence of biochemical losses in the general population is high, as compared to pregnancy losses that are confirmed after fetal activity [187,197]. This implies that the clinical pregnancy losses (visualized only) are less likely to occur due to chance alone [107].
- Number of previous miscarriages: According to ASRM[5] and ESHRE [33], two or more clinical pregnancy losses are included in the definition of RPL. The probability of having RM due to chance alone is decreased in women with a higher number of miscarriages [107].
- Products of conception (POC) analysis: RPL commonly occurs due to abnormal POC karyotype and fetal aneuploidy [10] thus appearing to occur due to chance. In contrast, MC with a normal POC karyotype is likely due to some other underlying pathology with a worse prognosis, subsequently leading to a higher number of RPLs in affected women [198,199].

Considering all these clinical factors, women with uRPL can be categorized into two groups (Table 3.2):

Type 1: older women with less than three biochemical losses or early pregnancy losses (occurring presumably by chance, i.e., with aneuploid POC). This group typically does not need any intervention and is considered to have a good prognosis.

Type 2: younger women with more than three clinically confirmed pregnancy losses (occurring presumably due to unidentified underlying pathology with normal POC results) [106,107].

Table 3.2 Clinical characteristics of women with Type 1 and Type 2 uRPL. Adapted from [106]

			Pregnancy Losses	
	Age	**Number**	**Types**	**Likely Pathology**
Type 1	Older	< 3	Includes biochemical	Abnormal karyotype of conceptus
Type 2	Younger	> 3	Clinical only	Remains unidentifiable

These criteria were used to define our cohort. Our preliminary data analysis showed that only a few women with exactly three biochemical pregnancy/non-visualized pregnancy losses had an abnormal karyotype of products of conception. Not many younger women with more than three clinical/visualized pregnancy losses were identified, although there was a high incidence of normal POC in both groups. Therefore, for our cohort, we defined Type 1 and Type 2 as follows: Older women (≥ 35 years) with only non-visualized pregnancy losses were categorized as Type 1, while younger women (< 35 years) with visualized and mixed (a combination of visualized and non-visualized) pregnancy losses were categorized as Type 2. Considering the two groups of uRPL women proposed by Saravelos and Li [107], we intended to examine any significant associations between the Types (Type 1 vs Type 2) and successful pregnancies (at ≥ 10 weeks' gestation).

3.2 Materials and methods

Participant selection

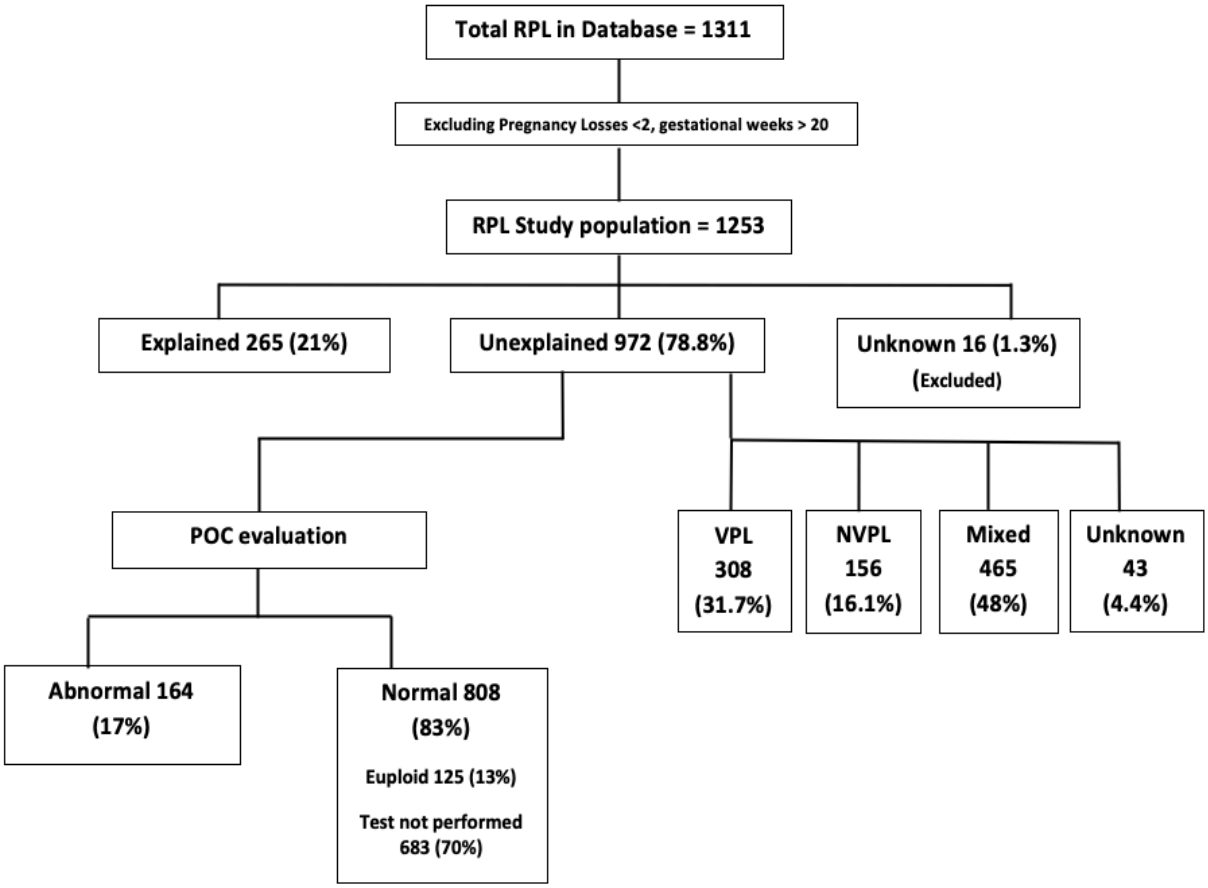
This retrospective study analyzed data from the same database as in Chapter 2, i.e., women referred to British Columbia's Hospital (BC Women's Hospital: BCWH) Center for RPL (a tertiary referral center), Vancouver British Columbia, Canada from January 1, 2011, to August 31, 2017. This study was approved by the University of British Columbia Research Ethics Board (Approval number: H13-03306). Data for the RPL registry is managed by the Research Electronic Data Capture (REDCap) data management platform at the BC Children's Research Institute (BCCHRI)[167].

According to ASRM guidelines, POC testing is not a part of routine evaluation done for uRPLs unless referred by the physician. For this study cohort of 972 women with uRPL (evaluated following the ASRM criteria), previous products of conception (POC) testing were reviewed after

referral. Among these women, 164 (17%) were found to have abnormal POC, 125 (13%) had normal POC, and 683 (70%) women did not have any POC testing. Cases in which POC was not performed and cases in which POC testing showed euploidy were both classified as "normal" (808) in the flow chart Figure 3.1. Because POC testing is not always possible (e.g, in NVPL), POC criteria were not included in the definition of Type 1 and Type 2 groups.

Following the European Society of Human Reproduction and Embryology (ESHRE) guidelines, we identified visualized, non-visualized and mixed types of pregnancy losses. Out of 972 women with uRPL, 308 (31.7%) were found to have only visualized pregnancy losses (VPL), 156 (16.1%) had only non-visualized pregnancy losses (NVPL), and 465 (48%) had a combination of both visualized and non-visualized pregnancy losses (Mixed) (Figure 3.1).

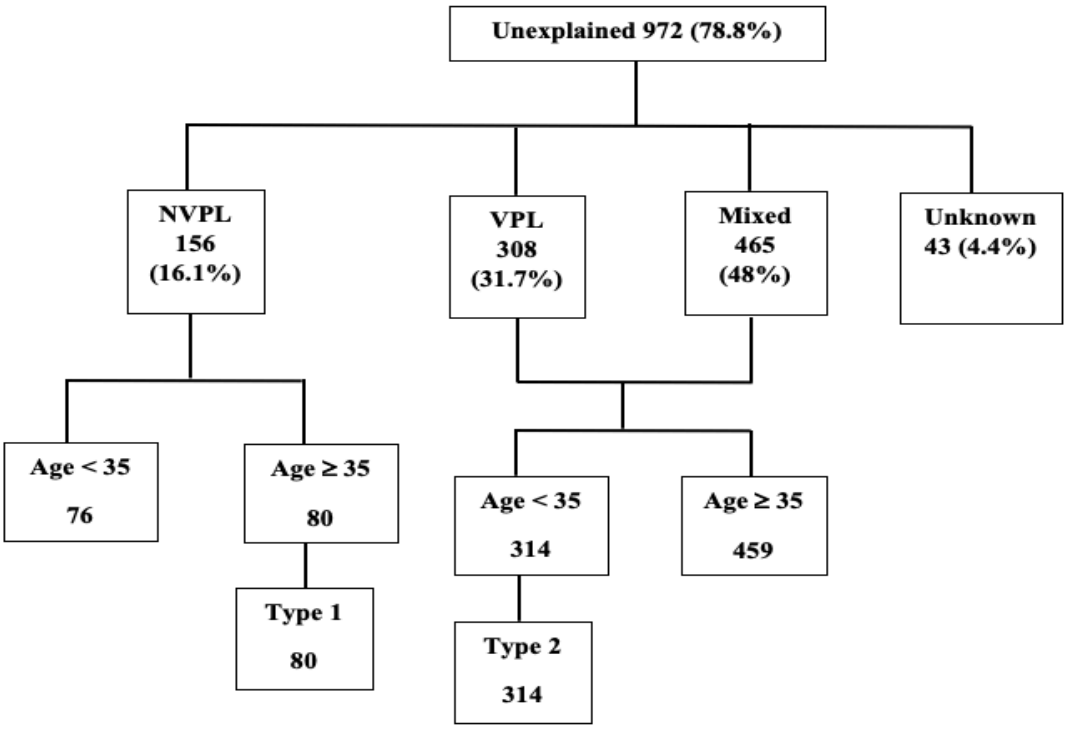
Figure 3.1 Participant selection flow chart.



Inclusion and exclusion criteria

Type 1 group included older women (≥35 years) with only non-visualized pregnancy losses, while Type 2 group included younger women (<35 years) with visualized and mixed pregnancy losses (a combination of visualized and non-visualized pregnancy loss). In the cohort of 972 uRPL women, 80 cases were Type 1 and 314 cases were Type 2; 572 cases were excluded as they did not match Type 1 or 2 criteria (Figure 3.2).

Figure 3.2 Flowchart showing division of Type 1 and Type 2 groups of uRPL women.



Data analysis

All analyses were performed using SPSS version 26.0 (SPSS Inc. Chicago, IL, USA). Descriptive analysis was carried out to evaluate demographic and clinical characteristics between two groups (Type 1 and 2). Bivariate analyses were performed to assess any significant differences in demographic and clinical factors between these groups. Chi-square test and Fisher's exact tests were used to compare categorical variables. A *p*-value of less than 0.05 was considered statistically significant.

Outcome

Successful pregnancy at or beyond 10 weeks of gestation, within 12 months of initial evaluation done at the RPL clinic.

Exposure of interest

Type 1 and Type 2 pregnancy loss (including age and type of prior pregnancy loss criteria).

Demographic and clinical factors

Clinical characteristics comprised the history of therapeutic abortions (reported as 0 vs 1 or ≥2), prior ectopic pregnancy (0 vs ≥1), prior pregnancy losses (< 3 vs ≥ 3), type of prior pregnancy losses (only VPL, only NVPL, Mixed), history of endometriosis, dysmenorrhea, dyspareunia, intermenstrual spotting, family history of RPL, alcohol consumption, smoking, and parity (0 vs. 1 or ≥2).

3.3 Results

Our objective was to assess the association between two different groups of women, i.e., Type 1 versus Type 2 group, and a successful pregnancy (at ≥ 10 weeks' gestation).

3.3.1 Descriptive statistics

Out of 972 uRPL women, 394 patients had the criteria of Type 1 and Type 2 groups. Older women (age ≥ 35 years) with a history of only NVPLs were categorized as Type 1 (8.2%), and younger women (age <35 years) with history of only VPL or both VPL and/or NVPL (mixed category) were classified as Type 2 (32.3%). Overall, 59.5% (578/972) women did not meet the criteria for Type 1 or Type 2. Most of the younger women had visualized and mixed types of pregnancy losses. Of note, 88% (275/314) of these women were found to have normal POC karyotypes and 12.4% (39/314) showed aneuploidy. In the Type 1 group (older women), there were 89% (71/80) euploid POC cases and 11.3% (9/80) were aneuploid (Table 3.3).

Table 3.3 Classification criteria for Type 1 and Type 2 uRPL women in our study.

Groups	Age	Types of pregnancy loss	POC		Total
			Normal	Abnormal	
Type 1	Older (≥ 35)	NVPL only	71 (89%)	9 (11.3%)	80
Type 2	Young (<35)	VPL and Mixed	275 (88%)	39 (12.4%)	314

We compared the demographic features of women with Type 1 vs Type 2. The proportion of women with mixed pregnancy losses (VPL and NVPL) was 47.2% (186/394), as compared with 32.5% of women with VPL (128/394) and 20.3% with NVPL (80/394). Some women in the Type 1 and Type 2 groups were reported to have a family history of RPL, intermenstrual spotting, history of dyspareunia, and endometriosis. Only 12.2% (48/394) had abnormal products of conception; 50.3% of women were regular alcohol drinkers. Other descriptive characteristics are presented in Table 3.4 and 3.5.

Table 3.4 Clinical characteristics of 394 women with uRPL in our cohort.

Variables	Categories	Frequencies	Percentages
Age	<35	314	79.7
	≥ 35	80	20.3
Total		394	100
Number of therapeutic abortions	0	318	80.7
	1	53	13.5
	≥ 2	23	5.8
Total		**394**	**100**
Prior ectopic pregnancy	No	369	93.7
	≥ 1	25	6.3
Total		**394**	**100**
Number of pregnancy losses	<3	167	42.4
	≥ 3	227	57.6
Total		**394**	**100**
Types of RPL	Visualized	128	32.5
	Non-Visualized	80	20.3
	Mixed	**186**	**47.2**
Total		394	100
History of endometriosis	No	342	93.4
	Yes	**24**	**6.6**
Total		366	100
Dysmenorrhea	No	234	62.2
	Yes	**142**	**37.8**
Total		376	100
Dyspareunia	No	303	80.6
	Yes	**73**	**19.4**
Total		376	100
Intermenstrual spotting	No	338	89.9
	Yes	**38**	**10.1**
Total		376	100
Family history of RPL	No	292	81.6
	Yes	**66**	**18.4**
Total		358	100
Products of conception	Normal	346	87.8
	Abnormal	**48**	**12.2**
Total		394	100
Alcohol	No	192	49.7
	Yes	194	50.3
Total		386	100
Smoking	No	353	91.7
	Yes	32	8.3
Total		**385**	**100**
Parity	0	250	63.5
	1	104	26.4
	≥2	**40**	**10.2**
Total		394	100

*Missing values for history of endometriosis = 28, dysmenorrhea = 18, dyspareunia = 18, intermenstrual spotting = 18, family history of RPL = 36, alcohol = 8 and smoking = 9.

Table 3.5 Outcomes of the 12-month follow-up with respect to subsequent pregnancy

Outcome variable	Categories		Frequencies	Percentages
Pregnancy	Miscarriage	< 10 weeks	145	36.8
	Ongoing pregnancy	≥ 10 weeks	190	48.2
	No pregnancy		59	15.0
Total			394	100

3.3.1.1 Bivariate analyses

We compared the demographic features between Type 1 and Type 2 groups of women with uRPL. Prior to bivariate analyses, the assumptions that variables were mutually exclusive and that atleast 80% of cells had a cell count of 5 or more were met.

Fisher's exact test and chi-square test were performed to examine the association between two groups of uRPL women, i.e. Type 1 and Type 2. Furthermore, the incidence of successful pregnancies (at ≥10 weeks of gestation) were analyzed among both the groups.

3.3.1.2 Association between clinical characteristics and Type 1 vs. Type 2 groups

A significant association was observed between the type of pregnancy loss (Type 1 and Type 2) and a history of endometriosis (p= 0.010) and parity (p= 0.009). Women in the Type 2 group were less likely to have a history of endometriosis. Among women in Type 2, 66.6% (209/314) of women were nulliparous, and among women in Type 1, 51.2% (41/80) of women were nulliparous. There were no differences between Type 1 and Type 2 groups with respect to the normal results for the products of conception evaluation (p= 0.474). There was no significant association seen for dysmenorrhea (p= 0.059) , dyspareunia (p= 0.0526), intermenstrual spotting (p= 0.336), family history of RPL (p= 0.061), therapeutic abortions (p= 0.39), ectopic pregnancies (p= 0.400), alcohol consumption (p= 0.202), smoking (p= 0.519) between both groups (Table 3.6).

Table 3.6 Differences in clinical characteristics between Type 1 and Type 2 uRPL groups.

Variables	Total	Type 1 (n=80)	Type 2 (n=314)	Chi-square & Fisher's Exact p-Value
History of endometriosis		n (%)	n (%)	
Yes	24	10 (13.7%)	14 (4.8%)	**0.010**
No	342	63 (86.3%)	279 (95.2%)	
Total		73 (100%)	293 (100%)	
Dysmenorrhea				
Yes	142	22 (29.3%)	120 (39.9%)	0.059
No	234	53 (70.7%)	181 (60.1%)	
Total		75 (100%)	301 (100%)	
Dyspareunia				
Yes	73	14 (18.9%)	59 (19.5%)	0.526
No	303	60 (81.1%)	243 (80.5%)	
Total		74 (100%)	302 (100%)	
Intermenstrual spotting				
Yes	38	9 (12.0%)	29 (9.6%)	0.336
No	338	66 (88.0%)	272 (90.4%)	
Total		75 (100%)	301 (100%)	
Family history of RPL				
Yes	66	8 (11.4%)	58 (20.1%)	0.061
No	292	62 (88.6%)	230 (79.9%)	
Total		70 (100%)	288 (100%)	
Therapeutic abortions				
No	318	61 (76.3%)	257 (81.8%)	0.39
1	53	12 (15.0%)	41 (13.1%)	
≥2	23	7 (8.8%)	16 (5.1%)	
Total		80 (100%)	314 (100%)	
Ectopic pregnancy				
No	369	76 (95.0%)	293 (93.3%)	0.400
≥1	25	4 (5.0%)	21 (6.7%)	
Total		80 (100%)	314 (100%)	
Smoking				
Yes	32	6 (7.7%)	26 (8.5%)	0.519
No	353	72 (92.3%)	281 (91.5%)	
Total		78 (100%)	307 (100%)	
Alcohol				
Yes	194	43 (55.1%)	151 (49.0%)	0.202
No	192	35 (44.9%)	157 (51.0%)	
Total		78 (100%)	308 (100%)	
Products of conception				
Normal	346	71 (88.8%)	275 (87.6%)	0.474
Abnormal	48	9 (11.3%)	39 (12.4%)	
Total		80 (100%)	314 (100%)	
Parity				
0	250	41 (51.2%)	209 (66.6%)	**0.009**
≥ 1	144	39 (48.8%)	105 (33.4%)	
Total		80 (100%)	314 (100%)	

Among the cohort of 394 uRPL women (Type 1 and Type 2), 84.3% (335/394) had subsequent pregnancies identified. Of these, 57% (190/335) had gone beyond 10 weeks' gestation and 43.2% (145/335) miscarried. There was a larger proportion of successful pregnancies among women in Type 2 group, 52.6% (165/314) as compared with 31.2% (25/80) among women in Type 1 uRPL (p-Value <0.001; Table 3.7).

Table 3.7 Successful pregnancies beyond 10 weeks' gestation among Type 1 and Type 2 category of uRPL women.

Pregnancy	Type 1	Type 2	Total	*pValue*
	N=80	N=314		
No pregnancy	14 (17.5%)	45 (14.3%)	59 (15.0%)	
< 10 weeks	41 (51.2%)	104 (33.1%)	145 (36.8%)	**<0.001**
≥ 10 weeks	25 (31.2%)	165 (52.6%)	190 (48.2%)	
Total	80	314	394	

3.3.2 Multivariate analysis

Multivariable analysis with respect to the associations with successful outcomes (an ongoing pregnancy at ≥10 weeks) was not performed because this was only an exploratory analysis with respect to a successful pregnancy following a pregnancy loss.

3.4 Discussion

In this retrospective study of confirmed unexplained recurrent pregnancy loss patients referred to our tertiary referral center, we found that among a cohort of 972 uRPL patients, only 8.2% (n=80) were categorized into Type 1 group and 32.3% (n=314) were categorized as Type 2 group of uRPL women. Older women (≥35 years) with a history of only preclinical pregnancy losses were classified as Type 1 group of uRPL women whereas, younger women (<35 years) with only clinical pregnancy losses and with a combination of clinical and preclinical losses were included in the Type 2 group. We found significant differences between type 1 and type 2 groups of uRPL women with respect to their history of endometriosis and parity. Parity is defined as the number of times a woman has given birth to a live-born or stillborn fetus. Regarding the analysis of products of conception, the majority of Type 1 and Type 2 groups of uRPL women had normal results.

Saravelos and Regan described Type 1 and Type 2 groups of uRPL in detail. According to this study, Type 1 women were older, experiencing less than three number of pregnancy losses, including biochemical losses, and were more likely to have aneuploidy in the products of conception [106]. On the other hand, Type 2 group women were younger, with more than three clinical pregnancy losses, and their products of conception had normal karyotyping. According to this review, Type 1 had a relatively good prognosis as they are thought to have no specific underlying pathology, whereas Type 2 women had poorer prognosis as they were more likely to suffer from some unidentified baseline pathology.

In our cohort we found that Type 1 women were older and were more likely to have a history of endometriosis and be parous in comparison to Type 2 group women.

Not many studies are known to show the correlation between endometriosis and uRPL. In a recent study done by Fox et al., 2019, the association between endometriosis and uRPL and unexplained infertility was studied by testing the level of BCL6 protein [200]. BCL6 is a protein found in the endometrium of women with a history of endometritis or who are infertile. Fox et al., 2019, found higher levels of BCL6 further leading to receptivity defects in the uRPL women in their cohort [200]. Although the proportion of women presenting with endometriosis was low in their study, larger studies might be needed to investigate further the role of endometriosis in uRPL women.

We also examined the proportion of successful pregnancies beyond 10 weeks, during a 12 months follow-up in both groups. We found that 85.0% (335/394) women in these two groups became pregnant within one year of being referred to our RPL clinic. Among Type 1 group of women who became pregnant, 38% (25/66) had a successful pregnancy (≥10 weeks' gestation proceeding beyond 10 weeks' gestation), while among Type 2 groups of women who became pregnant, the rate of successful pregnancy was 61.3% (165/269). Women in the Type 2 group were more likely to experience successful pregnancies going beyond 10 weeks of gestation. Maternal age is an important factor that contributes towards a successful pregnancy. As compared to the older women, younger women are more fertile and receptive towards implanting embryo. The majority of uRPL women, irrespective of their age or type of pregnancy loss were less likely to suffer from recurrent pregnancy loss by chance alone as they had normal results of products of conception analysis.

The strengths of this study include detailed clinical data collected into the standardized retrospective data registry and the follow-up of the cohort. Our study has several limitations. First, the cohort had a relatively small sample size, which was a consequence of the restrictive inclusion

criteria used to define the groups. The other limitations are similar to those mentioned in Chapter 2. Second, we did not have data about the ultimate pregnancy outcome, i.e., live birth. This is the ultimate optimal outcome in women with uRPL. Third, information about medical history was obtained by patients' recall and may not be completely accurate. Fourth, women attending our clinic may not be representative of all women with RPL. Thus, our results may not be directly generalizable to other populations.

Prior studies, including Clifford, Rai, Regan, 1997 [105], and some of the other studies [104,201], highlighted the importance of counseling and providing supportive care for the uRPL patients. These studies found a significant number of pregnancies resulting in live births among uRPL women who were referred to their clinic and underwent supportive care therapy. Overall 70% to 80% success rate with respect to live birth in the next pregnancy was achieved by this study group by providing TLC [105].

We did this exploratory study to examine the potential utility of classifying uRPL women into two distinct groups. The study started with a large cohort of 972 women with uRPL. A large number of women were excluded from the analysis as they did not satisfy the classification criteria for Type 1 or Type 2 uRPL women. We suggest initiating supportive management therapy during early pregnancy among the younger women with normal POC results. More research is needed to evaluate the importance of stratifying uRPL women according to the classification criteria suggested by Saravelos & Regan and Saravelos & Li [106,202].

Chapter 4: Conclusion and future direction

4.1 Conclusion

Unexplained recurrent pregnancy loss is a very distressing condition not only for the couples but also their healthcare providers. The cause of unexplained recurrent miscarriages is still unknown. Most women with RPL experience NVPL during their reproductive history. Clinicians do not routinely evaluate these patients as it is not recommended by the clinical guidelines [5,33]. We showed that many uRPL women have a history of NVPL and should be considered for clinical investigation. Investigation and treatment of these women during the period of early pregnancy losses could be helpful and might help investigators to discover new information regarding the causes of RPL.

In this thesis, I used a data registry from the tertiary referral center: British Columbia's Women's Hospital (BCWH), RPL clinic. The patients included in the study were women with uRPL.

Firstly, I found that there was a significantly greater number of uRPL women with a history of VPL resulting in successful pregnancies more than 10 weeks as compared to the women with a history of NVPL, after care and treatment provided at the BCWH. Women with NVPLs achieved 35.2% of ongoing pregnancies while VPL women achieved 45.4% of ongoing pregnancies in the whole cohort of uRPL. Another interesting finding was that uRPL women with NVPL's were more likely to undergo therapeutic abortions as compared to the clinically confirmed pregnancy losses. Therapeutic abortions and ectopic pregnancies are not considered in the diagnosis of RPL. However, an increased need for therapeutic abortions in early pregnancy in the RPL population is something worth investigating further to find out a possible underlying cause.

Our results with respect to the second study do not suggest major advantages of Type 1 and Type 2 classification of uRPL women. A large number of women were excluded from the analysis as they did not fit into the classification criteria (mix of Type 1 and Type 2 uRPL women). My findings suggest that women with uRPL should be evaluated irrespective of the age and type of pregnancy losses. Most of the clinically and non-clinically confirmed pregnancy losses were found to have normal findings on POC test analysis. This means that the majority of these women might be experiencing recurrent pregnancy losses due to some unidentified pathology that needs more clinical or laboratory investigations. Descriptive analysis of the individual groups showed that the proportion of women with a history of endometriosis was significantly lower among women with Type 2 group RPL (i.e., younger age, VPL, and Mixed type of pregnancy losses). Compared to Type 1 uRPL women, this is something that could be investigated further because the effect of diagnosing and treating endometriosis in younger women with uRPL might provide insight into the causes of RPL. Another interesting finding was that most of the women in Type 2 were parous. Parity is one of the important reproductive history factors that increases the chances of having another pregnancy reaching more than 10 weeks gestation [203]. Stratifying women with uRPL into two Type 1 and Type 2 groups largely restricted our study population. Therefore, a larger study is needed before using this classification as a prognostic tool for better outcomes.

4.2 Future direction

My research findings will provide new information for the clinicians and investigators towards better care for couples with unexplained recurrent pregnancy losses. Implementing new clinical guidelines with the inclusion of NVPLs for the investigation and treatment of uRPL patients would facilitate such endeavors. Further studies are needed to solidify the treatment plan for these women according to their identified etiologies. Ideally, prospective evaluation of patients with uRPL will mitigate the shortcomings of our study. We were approved for a prospective study (Ethics: H15-03466) and patient recruitment for this study is ongoing.

After validation, these findings should be shared among clinicians and the general population to spread awareness about early pregnancy loss. A thorough evaluation of non-clinical and clinical pregnancy losses can bring important new insights.

Finally, to share my research findings with patients and health care providers, I am going to present my findings at the ASRM 2020 Annual meeting. I will submit an abstract at the CFAS 2020 Annual Meeting, the Annual Academic Day of the Obstetrics and Gynaecology at UBC and will also submit manuscripts of my findings for publication.

References

1. Kolte AM, Bernardi LA, Christiansen OB, et al. Terminology for pregnancy loss prior to viability: A consensus statement from the ESHRE early pregnancy special interest group. *Hum Reprod*. 2015;30(3):495-498. doi: 10.1093/humrep/deu299 [doi].

2. Barnhart K, van Mello NM, Bourne T, et al. Pregnancy of unknown location: A consensus statement of nomenclature, definitions, and outcome. *Fertil Steril*. 2011;95(3):857-866. doi: 10.1016/j.fertnstert.2010.09.006 [doi].

3. Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. International committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary of ART terminology, 2009. *Fertil Steril*. 2009;92(5):1520-1524. doi: 10.1016/j.fertnstert.2009.09.009 [doi].

4. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. *N Engl J Med*. 1988;319(4):189-194. doi: 10.1056/NEJM198807283190401 [doi].

5. Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: A committee opinion. *Fertil Steril*. 2012;98(5):1103-1111. doi: 10.1016/j.fertnstert.2012.06.048 [doi].

6. Zinaman MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG. Estimates of human fertility and pregnancy loss. *Fertil Steril*. 1996;65(3):503-509. doi: S0015-0282(16)58144-8 [pii].

7. Wang X, Chen C, Wang L, Chen D, Guang W, French J. Conception, early pregnancy loss, and time to clinical pregnancy: A population-based prospective study. *Fertil Steril*. 2003;79(3):577-584. doi: S0015028202046940 [pii].

8. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: The 'black box' of early pregnancy loss. *Hum Reprod Update*. 2002;8(4):333-343. doi: 10.1093/humupd/8.4.333 [doi].

9. Larsen EC, Christiansen OB, Kolte AM, Macklon N. New insights into mechanisms behind miscarriage. *BMC Med*. 2013;11:154-154. doi: 10.1186/1741-7015-11-154 [doi].

10. Rai R, Regan L. Recurrent miscarriage. *Lancet*. 2006;368(9535):601-611. doi: S0140-6736(06)69204-0 [pii].

11. Grande M, Borrell A, Garcia-Posada R, et al. The effect of maternal age on chromosomal anomaly rate and spectrum in recurrent miscarriage. *Hum Reprod*. 2012;27(10):3109-3117. doi: 10.1093/humrep/des251 [doi].

12. Pellestor F, Andreo B, Arnal F, Humeau C, Demaille J. Maternal aging and chromosomal abnormalities: New data drawn from in vitro unfertilized human oocytes. *Hum Genet*. 2003;112(2):195-203. doi: 10.1007/s00439-002-0852-x [doi].

13. Enders AC. Trophoblast-uterine interactions in the first days of implantation: Models for the study of implantation events in the human. *Semin Reprod Med*. 2000;18(3):255-263. doi: 10.1055/s-2000-12563 [doi].

14. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. *N Engl J Med*. 2001;345(19):1400-1408. doi: 10.1056/NEJMra000763 [doi].

15. Pijnenborg R, Bland JM, Robertson WB, Dixon G, Brosens I. The pattern of interstitial trophoblastic invasion of the myometrium in early human pregnancy. *Placenta*. 1981;2(4):303-316. doi: S0143-4004(81)80027-6 [pii].

16. Pijnenborg R, Robertson WB, Brosens I, Dixon G. Review article: Trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. *Placenta*. 1981;2(1):71-91. doi: S0143-4004(81)80042-2 [pii].

17. HERTIG AT, ROCK J, ADAMS EC, MENKIN MC. Thirty-four fertilized human ova, good, bad and indifferent, recovered from 210 women of known fertility; a study of biologic wastage in early human pregnancy. *Pediatrics*. 1959;23(1 Part 2):202-211.

18. Lathi RB, Gray Hazard FK, Heerema-McKenney A, Taylor J, Chueh JT. First trimester miscarriage evaluation. *Semin Reprod Med*. 2011;29(6):463-469. doi: 10.1055/s-0031-1293200 [doi].

19. Houwert-de Jong MH, Bruinse HW, Eskes TK, Mantingh A, Termijtelen A, Kooyman CD. Early recurrent miscarriage: Histology of conception products. *Br J Obstet Gynaecol*. 1990;97(6):533-535. doi: 10.1111/j.1471-0528.1990.tb02525.x [doi].

20. Kohut KG, Anthony MN, Salafia CM. Decidual and placental histologic findings in patients experiencing spontaneous abortions in relation to pregnancy order. *Am J Reprod Immunol*. 1997;37(3):257-261. doi: 10.1111/j.1600-0897.1997.tb00224.x [doi].

21. Novak RW, Malone JM, Robinson HB. The role of the pathologist in the evaluation of first trimester abortions. *Pathol Annu*. 1990;25 Pt 1:297-311.

22. Psychoyos A. Uterine receptivity for nidation. *Ann N Y Acad Sci*. 1986;476:36-42. doi: 10.1111/j.1749-6632.1986.tb20920.x [doi].

23. Bergh PA, Navot D. The impact of embryonic development and endometrial maturity on the timing of implantation. *Fertil Steril*. 1992;58(3):537-542. doi: S0015-0282(16)55259-5 [pii].

24. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. *N Engl J Med*. 2001;345(19):1400-1408. doi: 10.1056/NEJMra000763 [doi].

25. Sebire NJ, Sepulveda W. Correlation of placental pathology with prenatal ultrasound findings. *J Clin Pathol*. 2008;61(12):1276-1284. doi: 10.1136/jcp.2008.055251 [doi].

26. Papaioannou GI, Syngelaki A, Maiz N, Ross JA, Nicolaides KH. Ultrasonographic prediction of early miscarriage. *Hum Reprod*. 2011;26(7):1685-1692. doi: 10.1093/humrep/der130 [doi].

27. Jauniaux E, Johns J, Burton GJ. The role of ultrasound imaging in diagnosing and investigating early pregnancy failure. *Ultrasound Obstet Gynecol*. 2005;25(6):613-624. doi: 10.1002/uog.1892 [doi].

28. Genest DR, Roberts D, Boyd T, Bieber FR. Fetoplacental histology as a predictor of karyotype: A controlled study of spontaneous first trimester abortions. *Hum Pathol*. 1995;26(2):201-209. doi: 0046-8177(95)90038-1 [pii].

29. Doubilet PM, Benson CB, Bourne T, et al. Diagnostic criteria for nonviable pregnancy early in the first trimester. *N Engl J Med*. 2013;369(15):1443-1451. doi: 10.1056/NEJMra1302417 [doi].

30. Jurkovic D, Overton C, Bender-Atik R. Diagnosis and management of first trimester miscarriage. *BMJ*. 2013;346:f3676. doi: 10.1136/bmj.f3676 [doi].

31. American College of Obstetricians and Gynecologists. ACOG practice bulletin. management of recurrent pregnancy loss. number 24, february 2001. (replaces technical bulletin number 212, september 1995). american college of obstetricians and gynecologists. *Int J Gynaecol Obstet*. 2002;78(2):179-190. doi: 10.1016/s0020-7292(02)00197-2 [doi].

32. Pinar MH, Gibbins K, He M, Kostadinov S, Silver R. Early pregnancy losses: Review of nomenclature, histopathology, and possible etiologies. *Fetal Pediatr Pathol*. 2018;37(3):191-209. doi: 10.1080/15513815.2018.1455775 [doi].

33. ESHRE Guideline Group on RPL, Bender Atik R, Christiansen OB, et al. ESHRE guideline: Recurrent pregnancy loss. *Hum Reprod Open*. 2018;2018(2):hoy004. doi: 10.1093/hropen/hoy004 [doi].

34. Kutteh WH. Novel strategies for the management of recurrent pregnancy loss. *Semin Reprod Med*. 2015;33(3):161-168. doi: 10.1055/s-0035-1552586 [doi].

35. Silver RM, Branch DW, Goldenberg R, Iams JD, Klebanoff MA. Nomenclature for pregnancy outcomes: Time for a change. *Obstet Gynecol*. 2011;118(6):1402-1408. doi: 10.1097/AOG.0b013e3182392977 [doi].

36. Silver RM, Branch DW, Goldenberg R, Iams JD, Klebanoff MA. Nomenclature for pregnancy outcomes: Time for a change. *Obstet Gynecol*. 2011;118(6):1402-1408. doi: 10.1097/AOG.0b013e3182392977 [doi].

37. Ford HB, Schust DJ. Recurrent pregnancy loss: Etiology, diagnosis, and therapy. *Rev Obstet Gynecol*. 2009;2(2):76-83.

38. Youssef A, Vermeulen N, Lashley, E E L O, Goddijn M, van der Hoorn, M L P. Comparison and appraisal of (inter)national recurrent pregnancy loss guidelines. *Reprod Biomed Online*. 2019;39(3):497-503. doi: S1472-6483(19)30385-2 [pii].

39. Stray-Pedersen B, Lorentzen-Styr AM. The prevalence of toxoplasma antibodies among 11,736 pregnant women in norway. *Scand J Infect Dis*. 1979;11(2):159-165. doi: 10.3109/inf.1979.11.issue-2.12 [doi].

40. Sugiura-Ogasawara M, Suzuki S, Ozaki Y, Katano K, Suzumori N, Kitaori T. Frequency of recurrent spontaneous abortion and its influence on further marital relationship and illness: The okazaki cohort study in japan. *J Obstet Gynaecol Res*. 2013;39(1):126-131. doi: 10.1111/j.1447-0756.2012.01973.x [doi].

41. Sugiura-Ogasawara M, Ozaki Y, Katano K, Suzumori N, Kitaori T, Mizutani E. Abnormal embryonic karyotype is the most frequent cause of recurrent miscarriage. *Hum Reprod*. 2012;27(8):2297-2303. doi: 10.1093/humrep/des179 [doi].

42. Werner M, Reh A, Grifo J, Perle MA. Characteristics of chromosomal abnormalities diagnosed after spontaneous abortions in an infertile population. *J Assist Reprod Genet*. 2012;29(8):817-820. doi: 10.1007/s10815-012-9781-3 [doi].

43. Laurino MY, Bennett RL, Saraiya DS, et al. Genetic evaluation and counseling of couples with recurrent miscarriage: Recommendations of the national society of genetic counselors. *J Genet Couns*. 2005;14(3):165-181. doi: 10.1007/s10897-005-3241-5 [doi].

44. De Braekeleer M, Dao TN. Cytogenetic studies in couples experiencing repeated pregnancy losses. *Hum Reprod*. 1990;5(5):519-528. doi: 10.1093/oxfordjournals.humrep.a137135 [doi].

45. Maithripala S, Durland U, Havelock J, et al. Prevalence and treatment choices for couples with recurrent pregnancy loss due to structural chromosomal anomalies. *Journal of Obstetrics and Gynaecology Canada*. 2018;40(6):655-662.

46. Barber JC, Cockwell AE, Grant E, Williams S, Dunn R, Ogilvie CM. Is karyotyping couples experiencing recurrent miscarriage worth the cost? *BJOG*. 2010;117(7):885-888. doi: 10.1111/j.1471-0528.2010.02566.x [doi].

47. Franssen MT, Korevaar JC, van der Veen F, Leschot NJ, Bossuyt PM, Goddijn M. Reproductive outcome after chromosome analysis in couples with two or more miscarriages: Index [corrected]-control study. *BMJ*. 2006;332(7544):759-763. doi: bmj.38735.459144.2F [pii].

48. Flynn H, Yan J, Saravelos SH, Li TC. Comparison of reproductive outcome, including the pattern of loss, between couples with chromosomal abnormalities and those with unexplained repeated miscarriages. *J Obstet Gynaecol Res*. 2014;40(1):109-116. doi: 10.1111/jog.12133 [doi].

49. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006;4(2):295-306. doi: JTH1753 [pii].

50. Andreoli L, Chighizola CB, Banzato A, Pons-Estel GJ, Ramire de Jesus G, Erkan D. Estimated frequency of antiphospholipid antibodies in patients with pregnancy morbidity, stroke, myocardial infarction, and deep vein thrombosis: A critical review of the literature. *Arthritis Care Res (Hoboken)*. 2013;65(11):1869-1873. doi: 10.1002/acr.22066 [doi].

51. de Jesus GR, Agmon-Levin N, Andrade CA, et al. 14th international congress on antiphospholipid antibodies task force report on obstetric antiphospholipid syndrome. *Autoimmun Rev*. 2014;13(8):795-813. doi: 10.1016/j.autrev.2014.02.003 [doi].

52. Opatrny L, David M, Kahn SR, Shrier I, Rey E. Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: A metaanalysis. *J Rheumatol*. 2006;33(11):2214-2221. doi: 06/13/106 [pii].

53. Meroni PL, Borghi MO, Raschi E, Tedesco F. Pathogenesis of antiphospholipid syndrome: Understanding the antibodies. *Nat Rev Rheumatol*. 2011;7(6):330-339. doi: 10.1038/nrrheum.2011.52 [doi].

54. Di Simone N, Castellani R, Caliandro D, Caruso A. Monoclonal anti-annexin V antibody inhibits trophoblast gonadotropin secretion and induces syncytiotrophoblast apoptosis. *Biol Reprod*. 2001;65(6):1766-1770. doi: 10.1095/biolreprod65.6.1766 [doi].

55. Di Simone N, Meroni PL, de Papa N, et al. Antiphospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered beta2-glycoprotein I. *Arthritis Rheum*. 2000;43(1):140-150. doi: 10.1002/1529-0131(200001)43:13.0.CO;2-P [doi].

56. de Jong PG, Goddijn M, Middeldorp S. Testing for inherited thrombophilia in recurrent miscarriage. *Semin Reprod Med*. 2011;29(6):540-547. doi: 10.1055/s-0031-1293207 [doi].

57. No RGG. The investigation and treatment of couples with recurrent first-trimester and second-trimester miscarriage. *RCOG: London, UK*. 2011.

58. Acien P, Acien M, Sanchez-Ferrer M. Complex malformations of the female genital tract. new types and revision of classification. *Hum Reprod*. 2004;19(10):2377-2384. doi: 10.1093/humrep/deh423 [doi].

59. Stephenson MD. Frequency of factors associated with habitual abortion in 197 couples. *Fertil Steril*. 1996;66(1):24-29. doi: S0015-0282(16)58382-4 [pii].

60. The american fertility society classifications of adnexal adhesions, distal tubal occlusion, tubal occlusion secondary to tubal ligation, tubal pregnancies, mullerian anomalies and intrauterine adhesions. *Fertil Steril*. 1988;49(6):944-955. doi: S0015-0282(16)59942-7 [pii].

61. Harger JH, Archer DF, Marchese SG, Muracca-Clemens M, Garver KL. Etiology of recurrent pregnancy losses and outcome of subsequent pregnancies. *Obstet Gynecol*. 1983;62(5):574-581.

62. Kobayashi A, Behringer RR. Developmental genetics of the female reproductive tract in mammals. *Nat Rev Genet*. 2003;4(12):969-980. doi: 10.1038/nrg1225 [doi].

63. Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman syndrome--one century later. *Fertil Steril*. 2008;89(4):759-779. doi: 10.1016/j.fertnstert.2008.02.096 [doi].

64. Alexander EK, Pearce EN, Brent GA, et al. 2017 guidelines of the american thyroid association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid*. 2017;27(3):315-389. doi: 10.1089/thy.2016.0457 [doi].

65. Vissenberg R, Manders VD, Mastenbroek S, et al. Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. *Hum Reprod Update*. 2015;21(3):378-387. doi: 10.1093/humupd/dmv004 [doi].

66. van den Boogaard E, Vissenberg R, Land JA, et al. Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: A systematic review. *Hum Reprod Update*. 2011;17(5):605-619. doi: 10.1093/humupd/dmr024 [doi].

67. Haddow JE, Palomaki GE, Allan WC, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N Engl J Med*. 1999;341(8):549-555. doi: 10.1056/NEJM199908193410801 [doi].

68. Abalovich M, Gutierrez S, Alcaraz G, Maccallini G, Garcia A, Levalle O. Overt and subclinical hypothyroidism complicating pregnancy. *Thyroid*. 2002;12(1):63-68. doi: 10.1089/105072502753451986 [doi].

69. Taylor PN, Minassian C, Rehman A, et al. TSH levels and risk of miscarriage in women on long-term levothyroxine: A community-based study. *J Clin Endocrinol Metab*. 2014;99(10):3895-3902. doi: 10.1210/jc.2014-1954 [doi].

70. McQueen DB, Perfetto CO, Hazard FK, Lathi RB. Pregnancy outcomes in women with chronic endometritis and recurrent pregnancy loss. *Fertil Steril*. 2015;104(4):927-931. doi: S0015-0282(15)00473-2 [pii].

71. Boots CE, Bernardi LA, Stephenson MD. Frequency of euploid miscarriage is increased in obese women with recurrent early pregnancy loss. *Fertil Steril*. 2014;102(2):455-459. doi: 10.1016/j.fertnstert.2014.05.005 [doi].

72. Zidi-Jrah I, Hajlaoui A, Mougou-Zerelli S, et al. Relationship between sperm aneuploidy, sperm DNA integrity, chromatin packaging, traditional semen parameters, and recurrent pregnancy loss. *Fertil Steril*. 2016;105(1):58-64. doi: 10.1016/j.fertnstert.2015.09.041 [doi].

73. Carrell DT, Liu L, Peterson CM, et al. Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. *Arch Androl*. 2003;49(1):49-55. doi: 10.1080/01485010290099390 [doi].

74. Hill JA, Abbott AF, Politch JA. Sperm morphology and recurrent abortion. *Fertil Steril*. 1994;61(4):776-778. doi: S0015-0282(16)56661-8 [pii].

75. Carrell DT, Wilcox AL, Lowy L, et al. Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. *Obstet Gynecol*. 2003;101(6):1229-1235. doi: S0029784403003399 [pii].

76. Kesmodel U, Wisborg K, Olsen SF, Henriksen TB, Secher NJ. Moderate alcohol intake in pregnancy and the risk of spontaneous abortion. *Alcohol Alcohol*. 2002;37(1):87-92. doi: 10.1093/alcalc/37.1.87 [doi].

77. Ness RB, Grisso JA, Hirschinger N, et al. Cocaine and tobacco use and the risk of spontaneous abortion. *N Engl J Med*. 1999;340(5):333-339. doi: 10.1056/NEJM199902043400501 [doi].

78. Khalife D, Ghazeeri G, Kutteh W. Review of current guidelines for recurrent pregnancy loss: New strategies for optimal evaluation of women who may be superfertile. *Semin Perinatol*. 2019;43(2):105-115. doi: S0146-0005(18)30144-7 [pii].

79. Jauniaux E, Farquharson RG, Christiansen OB, Exalto N. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. *Hum Reprod*. 2006;21(9):2216-2222. doi: del150 [pii].

80. Christiansen OB. Evidence-based investigations and treatments of recurrent pregnancy loss. *Curr Opin Obstet Gynecol*. 2006;18(3):304-312. doi: 10.1097/01.gco.0000193011.73405.07 [doi].

81. Bernardi LA, Plunkett BA, Stephenson MD. Is chromosome testing of the second miscarriage cost saving? A decision analysis of selective versus universal recurrent pregnancy loss evaluation. *Fertil Steril*. 2012;98(1):156-161. doi: 10.1016/j.fertnstert.2012.03.038 [doi].

82. Jaslow CR, Carney JL, Kutteh WH. Diagnostic factors identified in 1020 women with two versus three or more recurrent pregnancy losses. *Fertil Steril*. 2010;93(4):1234-1243. doi: 10.1016/j.fertnstert.2009.01.166 [doi].

83. Foyouzi N, Cedars MI, Huddleston HG. Cost-effectiveness of cytogenetic evaluation of products of conception in the patient with a second pregnancy loss. *Fertil Steril*. 2012;98(1):151-155. doi: 10.1016/j.fertnstert.2012.04.007 [doi].

84. Dahdouh EM, Balayla J, Garcia-Velasco JA. Preimplantation genetic screening using comprehensive chromosome screening: Evidence and remaining challenges. *Hum Reprod*. 2015;30(6):1515-1516. doi: 10.1093/humrep/dev079 [doi].

85. Rosenfeld JA, Tucker ME, Escobar LF, et al. Diagnostic utility of microarray testing in pregnancy loss. *Ultrasound in Obstetrics & Gynecology*. 2015;46(4):478-486.

86. Wang BT, Chong TP, Boyar FZ, et al. Abnormalities in spontaneous abortions detected by G-banding and chromosomal microarray analysis (CMA) at a national reference laboratory. *Molecular cytogenetics*. 2014;7(1):33.

87. Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med*. 2012;367(23):2175-2184.

88. Li G, Jin H, Xin Z, et al. Increased IVF pregnancy rates after microarray preimplantation genetic diagnosis due to parental translocations. *Syst Biol Reprod Med*. 2014;60(2):119-124. doi: 10.3109/19396368.2013.875241 [doi].

89. Tobler KJ, Brezina PR, Benner AT, Du L, Xu X, Kearns WG. Two different microarray technologies for preimplantation genetic diagnosis and screening, due to reciprocal translocation imbalances, demonstrate equivalent euploidy and clinical pregnancy rates. *J Assist Reprod Genet*. 2014;31(7):843-850. doi: 10.1007/s10815-014-0230-3 [doi].

90. Brezina PR, Kearns WG. The evolving role of genetics in reproductive medicine. *Obstet Gynecol Clin North Am*. 2014;41(1):41-55. doi: 10.1016/j.ogc.2013.10.006 [doi].

91. Madan K, Nieuwint AW, van Bever Y. Recombination in a balanced complex translocation of a mother leading to a balanced reciprocal translocation in the child. review of 60 cases of balanced complex translocations. *Hum Genet*. 1997;99(6):806-815. https://pubmed.ncbi.nlm.nih.gov/9187678. doi: 10.1007/s004390050453.

92. Dhillon RK, Hillman SC, Morris RK, et al. Additional information from chromosomal microarray analysis (CMA) over conventional karyotyping when diagnosing chromosomal abnormalities in miscarriage: A systematic review and meta-analysis. *BJOG*. 2014;121(1):11-21. doi: 10.1111/1471-0528.12382 [doi].

93. Popescu F, Jaslow CR, Kutteh WH. Recurrent pregnancy loss evaluation combined with 24-chromosome microarray of miscarriage tissue provides a probable or definite cause of pregnancy loss in over 90% of patients. *Hum Reprod*. 2018;33(4):579-587. doi: 10.1093/humrep/dey021 [doi].

94. Colley E, Hamilton S, Smith P, Morgan NV, Coomarasamy A, Allen S. Potential genetic causes of miscarriage in euploid pregnancies: A systematic review. *Hum Reprod Update*. 2019;25(4):452-472. doi: 10.1093/humupd/dmz015 [doi].

95. Abalovich M, Amino N, Barbour LA, et al. Management of thyroid dysfunction during pregnancy and postpartum: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2007;92(8 Suppl):1. doi: 10.1210/jc.2007-0141 [doi].

96. Negro R, Schwartz A, Gismondi R, Tinelli A, Mangieri T, Stagnaro-Green A. Increased pregnancy loss rate in thyroid antibody negative women with TSH levels between 2.5 and 5.0 in the first trimester of pregnancy. *J Clin Endocrinol Metab*. 2010;95(9):44. doi: 10.1210/jc.2010-0340 [doi].

97. Greene MF, Hare JW, Cloherty JP, Benacerraf BR, Soeldner JS. First-trimester hemoglobin A1 and risk for major malformation and spontaneous abortion in diabetic pregnancy. *Teratology*. 1989;39(3):225-231. doi: 10.1002/tera.1420390303 [doi].

98. El Hachem H, Crepaux V, May-Panloup P, Descamps P, Legendre G, Bouet PE. Recurrent pregnancy loss: Current perspectives. *Int J Womens Health*. 2017;9:331-345. doi: 10.2147/IJWH.S100817 [doi].

99. Stephenson MD, Sierra S. Reproductive outcomes in recurrent pregnancy loss associated with a parental carrier of a structural chromosome rearrangement. *Hum Reprod*. 2006;21(4):1076-1082. doi: dei417 [pii].

100. El Hachem H, Crepaux V, May-Panloup P, Descamps P, Legendre G, Bouet PE. Recurrent pregnancy loss: Current perspectives. *Int J Womens Health*. 2017;9:331-345. doi: 10.2147/IJWH.S100817 [doi].

101. Brezina PR, Kutteh WH. Classic and cutting-edge strategies for the management of early pregnancy loss. *Obstet Gynecol Clin North Am*. 2014;41(1):1-18. doi: 10.1016/j.ogc.2013.10.011 [doi].

102. Li TC, Iqbal T, Anstie B, et al. An analysis of the pattern of pregnancy loss in women with recurrent miscarriage. *Fertil Steril*. 2002;78(5):1100-1106. doi: S0015028202042073 [pii].

103. Clifford K, Rai R, Regan L. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. *Hum Reprod*. 1997;12(2):387-389. doi: 10.1093/humrep/12.2.387 [doi].

104. Stray-Pedersen B, Stray-Pedersen S. Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. *Am J Obstet Gynecol*. 1984;148(2):140-146. doi: S0002-9378(84)80164-7 [pii].

105. Clifford K, Rai R, Regan L. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. *Hum Reprod*. 1997;12(2):387-389. doi: 10.1093/humrep/12.2.387 [doi].

106. Saravelos SH, Regan L. Unexplained recurrent pregnancy loss. *Obstet Gynecol Clin North Am*. 2014;41(1):157-166. doi: 10.1016/j.ogc.2013.10.008 [doi].

107. Saravelos SH, Li TC. Unexplained recurrent miscarriage: How can we explain it? *Hum Reprod*. 2012;27(7):1882-1886. doi: 10.1093/humrep/des102 [doi].

108. Remohi J, Gallardo E, Levy M, et al. Oocyte donation in women with recurrent pregnancy loss. *Hum Reprod*. 1996;11(9):2048-2051. doi: 10.1093/oxfordjournals.humrep.a019541 [doi].

109. Vissenberg R, Goddijn M. Is there a role for assisted reproductive technology in recurrent miscarriage? *Semin Reprod Med*. 2011;29(6):548-556. https://pubmed.ncbi.nlm.nih.gov/22161467. doi: 10.1055/s-0031-1293208.

110. Robinson L, Gallos ID, Conner SJ, et al. The effect of sperm DNA fragmentation on miscarriage rates: A systematic review and meta-analysis. *Hum Reprod*. 2012;27(10):2908-2917. doi: 10.1093/humrep/des261 [doi].

111. Musters AM, Repping S, Korevaar JC, et al. Pregnancy outcome after preimplantation genetic screening or natural conception in couples with unexplained recurrent miscarriage: A systematic review of the best available evidence. *Fertil Steril*. 2011;95(6):2153-2157.e21573. https://pubmed.ncbi.nlm.nih.gov/21215967. doi: 10.1016/j.fertnstert.2010.12.022.

112. Teklenburg G, Salker M, Molokhia M, et al. Natural selection of human embryos: Decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. *PLoS One*. 2010;5(4):e10258. doi: 10.1371/journal.pone.0010258 [doi].

113. Salker M, Teklenburg G, Molokhia M, et al. Natural selection of human embryos: Impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. *PLoS One*. 2010;5(4):e10287. doi: 10.1371/journal.pone.0010287 [doi].

114. Weimar CH, Kavelaars A, Brosens JJ, et al. Endometrial stromal cells of women with recurrent miscarriage fail to discriminate between high- and low-quality human embryos. *PLoS One*. 2012;7(7):e41424. doi: 10.1371/journal.pone.0041424 [doi].

115. Coulam C. What about superfertility, decidualization, and natural selection? *J Assist Reprod Genet*. 2016;33(5):577-580. doi: 10.1007/s10815-016-0658-8 [doi].

116. Alexander EK, Marqusee E, Lawrence J, Jarolim P, Fischer GA, Larsen PR. Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. *N Engl J Med*. 2004;351(3):241-249. doi: 10.1056/NEJMoa040079 [doi].

117. Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. *Endocr Rev*. 2010;31(5):702-755. doi: 10.1210/er.2009-0041 [doi].

118. Glinoer D. The regulation of thyroid function in pregnancy: Pathways of endocrine adaptation from physiology to pathology. *Endocr Rev*. 1997;18(3):404-433. doi: 10.1210/edrv.18.3.0300 [doi].

119. Negro R, Formoso G, Mangieri T, Pezzarossa A, Dazzi D, Hassan H. Levothyroxine treatment in euthyroid pregnant women with autoimmune thyroid disease: Effects on obstetrical complications. *J Clin Endocrinol Metab*. 2006;91(7):2587-2591. doi: jc.2005-1603 [pii].

120. De Groot L, Abalovich M, Alexander EK, et al. Management of thyroid dysfunction during pregnancy and postpartum: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2012;97(8):2543-2565. doi: 10.1210/jc.2011-2803 [doi].

121. Smith ML, Schust DJ. Endocrinology and recurrent early pregnancy loss. *Semin Reprod Med*. 2011;29(6):482-490. doi: 10.1055/s-0031-1293202 [doi].

122. Jakubowicz DJ, Iuorno MJ, Jakubowicz S, Roberts KA, Nestler JE. Effects of metformin on early pregnancy loss in the polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2002;87(2):524-529. doi: 10.1210/jcem.87.2.8207 [doi].

123. Glueck CJ, Phillips H, Cameron D, Sieve-Smith L, Wang P. Continuing metformin throughout pregnancy in women with polycystic ovary syndrome appears to safely reduce first-trimester spontaneous abortion: A pilot study. *Fertil Steril*. 2001;75(1):46-52.

124. Tang A, Alfirevic Z, Quenby S. Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: A systematic review. *Human Reproduction*. 2011;26(8):1971-1980.

125. Mastenbroek S, Repping S. Preimplantation genetic screening: Back to the future. *Hum Reprod*. 2014;29(9):1846-1850. doi: 10.1093/humrep/deu163 [doi].

126. Murugappan G, Shahine LK, Perfetto CO, Hickok LR, Lathi RB. Intent to treat analysis of in vitro fertilization and preimplantation genetic screening versus expectant management in patients with recurrent pregnancy loss. *Hum Reprod*. 2016;31(8):1668-1674. doi: 10.1093/humrep/dew135 [doi].

127. Shahine LK, Lathi RB. Embryo selection with preimplantation chromosomal screening in patients with recurrent pregnancy loss. *Semin Reprod Med*. 2014;32(2):93-99. doi: 10.1055/s-0033-1363550 [doi].

128. Mowbray JF, Gibbings C, Liddell H, Reginald PW, Underwood JL, Beard RW. Controlled trial of treatment of recurrent spontaneous abortion by immunisation with paternal cells. *Lancet*. 1985;1(8435):941-943. doi: S0140-6736(85)91723-4 [pii].

129. Beer AE, Quebbeman JF, Ayers JW, Haines RF. Major histocompatibility complex antigens, maternal and paternal immune responses, and chronic habitual abortions in humans. *Am J Obstet Gynecol*. 1981;141(8):987-999. doi: S0002-9378(16)32690-4 [pii].

130. Christiansen OB, Mathiesen O, Husth M, Lauritsen JG, Grunnet N. Placebo-controlled trial of active immunization with third party leukocytes in recurrent miscarriage. *Acta Obstet Gynecol Scand*. 1994;73(3):261-268. doi: 10.3109/00016349409023451 [doi].

131. Wang SW, Zhong SY, Lou LJ, Hu ZF, Sun HY, Zhu HY. The effect of intravenous immunoglobulin passive immunotherapy on unexplained recurrent spontaneous abortion: A meta-analysis. *Reprod Biomed Online*. 2016;33(6):720-736. doi: S1472-6483(16)30509-0 [pii].

132. Kling C, Steinmann J, Westphal E, Magez J, Kabelitz D. Adverse effects of intradermal allogeneic lymphocyte immunotherapy: Acute reactions and role of autoimmunity. *Hum Reprod*. 2005;21(2):429-435. https://doi.org/10.1093/humrep/dei316. doi: 10.1093/humrep/dei316.

133. Egerup P, Lindschou J, Gluud C, Christiansen OB, ImmuReM IPD Study Group. The effects of intravenous immunoglobulins in women with recurrent miscarriages: A systematic review of randomised trials with meta-analyses and trial sequential analyses including individual patient data. *PLoS One*. 2015;10(10):e0141588. doi: 10.1371/journal.pone.0141588 [doi].

134. Hutton B, Sharma R, Fergusson D, et al. Use of intravenous immunoglobulin for treatment of recurrent miscarriage: A systematic review. *BJOG*. 2007;114(2):134-142. doi: BJO1201 [pii].

135. Gomaa MF, Elkholy AG, El-Said MM, Abdel-Salam NE. Combined oral prednisolone and heparin versus heparin: The effect on peripheral NK cells and clinical outcome in patients with

unexplained recurrent miscarriage. A double-blind placebo randomized controlled trial. *Arch Gynecol Obstet*. 2014;290(4):757-762. doi: 10.1007/s00404-014-3262-0 [doi].

136. Laskin CA, Bombardier C, Hannah ME, et al. Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. *N Engl J Med*. 1997;337(3):148-154. https://doi.org/10.1056/NEJM199707173370302. doi: 10.1056/NEJM199707173370302.

137. Pasquier E, de Saint Martin L, Bohec C, et al. Enoxaparin for prevention of unexplained recurrent miscarriage: A multicenter randomized double-blind placebo-controlled trial. *Blood*. 2015;125(14):2200-2205. doi: 10.1182/blood-2014-11-610857 [doi].

138. Schleussner E, Petroff D. Low-molecular-weight heparin for women with unexplained recurrent pregnancy loss. *Ann Intern Med*. 2015;163(6):485-3. doi: 10.7326/L15-5137-3 [doi].

139. de Jong PG, Kaandorp S, Di Nisio M, Goddijn M, Middeldorp S. Aspirin and/or heparin for women with unexplained recurrent miscarriage with or without inherited thrombophilia. *Cochrane Database of Systematic Reviews*. 2014(7). https://doi.org//10.1002/14651858.CD004734.pub4. doi: 10.1002/14651858.CD004734.pub4.

140. Shaaban OM, Abbas AM, Zahran KM, Fathalla MM, Anan MA, Salman SA. Low-molecular-weight heparin for the treatment of unexplained recurrent miscarriage with negative antiphospholipid antibodies: A randomized controlled trial. *Clin Appl Thromb Hemost*. 2017;23(6):567-572. https://doi.org/10.1177/1076029616665167. doi: 10.1177/1076029616665167.

141. Selhub J, Rosenberg IH. Excessive folic acid intake and relation to adverse health outcome. *Biochimie*. 2016;126:71-78. doi: 10.1016/j.biochi.2016.04.010 [doi].

142. Kumar A, Begum N, Prasad S, Aggarwal S, Sharma S. Oral dydrogesterone treatment during early pregnancy to prevent recurrent pregnancy loss and its role in modulation of cytokine production: A double-blind, randomized, parallel, placebo-controlled trial. *Fertil Steril*. 2014;102(5):1357-1363.e3. doi: 10.1016/j.fertnstert.2014.07.1251 [doi].

143. Coomarasamy A, Williams H, Truchanowicz E, et al. A randomized trial of progesterone in women with recurrent miscarriages. *N Engl J Med*. 2015;373(22):2141-2148. doi: 10.1056/NEJMoa1504927 [doi].

144. Saccone G, Schoen C, Franasiak JM, Scott RT, Berghella V. Supplementation with progestogens in the first trimester of pregnancy to prevent miscarriage in women with unexplained recurrent miscarriage: A systematic review and meta-analysis of randomized, controlled trials. *Fertil Steril*. 2017;107(2):430-438.e3. doi: S0015-0282(16)62954-0 [pii].

145. Mayer K, Meyer S, Reinholz-Muhly M, et al. Short-time infusion of fish oil-based lipid emulsions, approved for parenteral nutrition, reduces monocyte proinflammatory cytokine generation and adhesive interaction with endothelium in humans. *J Immunol*. 2003;171(9):4837-4843. doi: 10.4049/jimmunol.171.9.4837 [doi].

146. Granato D, Blum S, Rossle C, Le Boucher J, Malnoe A, Dutot G. Effects of parenteral lipid emulsions with different fatty acid composition on immune cell functions in vitro. *JPEN J Parenter Enteral Nutr*. 2000;24(2):113-118. doi: 10.1177/0148607100024002113 [doi].

147. Roussev RG, Acacio B, Ng SC, Coulam CB. Duration of intralipid's suppressive effect on NK cell's functional activity. *Am J Reprod Immunol*. 2008;60(3):258-263. doi: 10.1111/j.1600-0897.2008.00621.x [doi].

148. Meng L, Lin J, Chen L, et al. Effectiveness and potential mechanisms of intralipid in treating unexplained recurrent spontaneous abortion. *Arch Gynecol Obstet*. 2016;294(1):29-39. doi: 10.1007/s00404-015-3922-8 [doi].

149. Jokhi PP, King A, Loke YW. Production of granulocyte-macrophage colony-stimulating factor by human trophoblast cells and by decidual large granular lymphocytes. *Hum Reprod*. 1994;9(9):1660-1669. doi: 10.1093/oxfordjournals.humrep.a138769 [doi].

150. Scarpellini F, Sbracia M. Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: A randomised controlled trial. *Hum Reprod*. 2009;24(11):2703-2708. doi: 10.1093/humrep/dep240 [doi].

151. Santjohanser C, Knieper C, Franz C, et al. Granulocyte-colony stimulating factor as treatment option in patients with recurrent miscarriage. *Arch Immunol Ther Exp (Warsz)*. 2013;61(2):159-164. doi: 10.1007/s00005-012-0212-z [doi].

152. van Hoogenhuijze NE, Kasius JC, Broekmans FJM, Bosteels J, Torrance HL. Endometrial scratching prior to IVF; does it help and for whom? A systematic review and meta-analysis. *Hum Reprod Open*. 2019;2019(1):hoy025. doi: 10.1093/hropen/hoy025 [doi].

153. Jauniaux E, Burton GJ. Pathophysiology of histological changes in early pregnancy loss. *Placenta*. 2005;26(2-3):114-123. doi: S0143400404001560 [pii].

154. Pandya PP, Snijders RJ, Psara N, Hilbert L, Nicolaides KH. The prevalence of non-viable pregnancy at 10-13 weeks of gestation. *Ultrasound Obstet Gynecol*. 1996;7(3):170-173. doi: 10.1046/j.1469-0705.1996.07030170.x [doi].

155. American College of Obstetricians and Gynecologists. ACOG practice bulletin. management of recurrent pregnancy loss. number 24, february 2001. (replaces technical bulletin number 212, september 1995). american college of obstetricians and gynecologists. *Int J Gynaecol Obstet*. 2002;78(2):179-190. doi: 10.1016/s0020-7292(02)00197-2 [doi].

156. Massalska D, Zimowski JG, Bijok J, et al. First trimester pregnancy loss: Clinical implications of genetic testing. *J Obstet Gynaecol Res*. 2017;43(1):23-29. doi: 10.1111/jog.13179 [doi].

157. Goddijn M, Leschot NJ. Genetic aspects of miscarriage. *Baillieres Best Pract Res Clin Obstet Gynaecol*. 2000;14(5):855-865. doi: 10.1053/beog.2000.0124 [doi].

158. De Braekeleer M, Dao TN. Cytogenetic studies in couples experiencing repeated pregnancy losses. *Hum Reprod*. 1990;5(5):519-528. doi: 10.1093/oxfordjournals.humrep.a137135 [doi].

159. Kirk E, Condous G, Bourne T. Pregnancies of unknown location. *Best Pract Res Clin Obstet Gynaecol*. 2009;23(4):493-499. doi: 10.1016/j.bpobgyn.2009.01.008 [doi].

160. Hahlin M, Thorburn J, Bryman I. The expectant management of early pregnancies of uncertain site. *Hum Reprod*. 1995;10(5):1223-1227. doi: 10.1093/oxfordjournals.humrep.a136123 [doi].

161. Banerjee S, Aslam N, Zosmer N, Woelfer B, Jurkovic D. The expectant management of women with early pregnancy of unknown location. *Ultrasound Obstet Gynecol*. 1999;14(4):231-236. doi: 10.1046/j.1469-0705.1999.14040231.x [doi].

162. Banerjee S, Aslam N, Woelfer B, Lawrence A, Elson J, Jurkovic D. Expectant management of early pregnancies of unknown location: A prospective evaluation of methods to predict spontaneous resolution of pregnancy. *BJOG*. 2001;108(2):158-163. doi: 10.1111/j.1471-0528.2001.00031.x [doi].

163. Kirk E, Condous G, Van Calster B, Van Huffel S, Timmerman D, Bourne T. Rationalizing the follow-up of pregnancies of unknown location. *Hum Reprod*. 2007;22(6):1744-1750. doi: dem073 [pii].

164. Kolte AM, van Oppenraaij RH, Quenby S, et al. Non-visualized pregnancy losses are prognostically important for unexplained recurrent miscarriage. *Hum Reprod*. 2014;29(5):931-937. doi: 10.1093/humrep/deu042 [doi].

165. Wilcox AJ, Baird DD, Weinberg CR, et al. The use of biochemical assays in epidemiologic studies of reproduction. *Environ Health Perspect*. 1987;75:29-35. doi: 10.1289/ehp.877529 [doi].

166. Saravelos SH, Li TC. Unexplained recurrent miscarriage: How can we explain it? *Hum Reprod*. 2012;27(7):1882-1886. doi: 10.1093/humrep/des102 [doi].

167. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing

translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi: 10.1016/j.jbi.2008.08.010 [doi].

168. Kolte AM, Bernardi LA, Christiansen OB, et al. Terminology for pregnancy loss prior to viability: A consensus statement from the ESHRE early pregnancy special interest group. *Hum Reprod*. 2015;30(3):495-498. doi: 10.1093/humrep/deu299 [doi].

169. Committee on Practice Bulletins-Gynecology. The american college of obstetricians and gynecologists practice bulletin no. 150. early pregnancy loss. *Obstet Gynecol*. 2015;125(5):1258-1267. doi: 10.1097/01.AOG.0000465191.27155.25 [doi].

170. American College of Obstetricians and Gynecologists Committee on Practice Bulletins-Obstetrics. ACOG practice bulletin no. 118: Antiphospholipid syndrome. *Obstet Gynecol*. 2011;117(1):192-199. doi: 10.1097/AOG.0b013e31820a61f9 [doi].

171. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006;4(2):295-306. doi: JTH1753 [pii].

172. Greenberg T, Tzivian L, Harlev A, Serjienko R, Mazor M, Bashiri A. Index pregnancy versus post-index pregnancy in patients with recurrent pregnancy loss. *J Matern Fetal Neonatal Med*. 2015;28(1):63-67. doi: 10.3109/14767058.2014.900752 [doi].

173. Shapira E, Ratzon R, Shoham-Vardi I, Serjienko R, Mazor M, Bashiri A. Primary vs. secondary recurrent pregnancy loss--epidemiological characteristics, etiology, and next pregnancy outcome. *J Perinat Med*. 2012;40(4):389-396. doi: 10.1515/jpm-2011-0315 [doi].

174. Sheiner E, Levy A, Katz M, Mazor M. Pregnancy outcome following recurrent spontaneous abortions. *Eur J Obstet Gynecol Reprod Biol*. 2005;118(1):61-65. doi: S0301-2115(04)00352-5 [pii].

175. De Wals P, Tairou F, Van Allen MI, et al. Reduction in neural-tube defects after folic acid fortification in canada. *N Engl J Med*. 2007;357(2):135-142. doi: 357/2/135 [pii].

176. Pitkin RM. Folate and neural tube defects. *Am J Clin Nutr*. 2007;85(1):285S-288S. doi: 85/1/285S [pii].

177. Gaskins AJ, Rich-Edwards JW, Hauser R, et al. Maternal prepregnancy folate intake and risk of spontaneous abortion and stillbirth. *Obstet Gynecol*. 2014;124(1):23-31. doi: 10.1097/AOG.0000000000000343 [doi].

178. Barnhart K, van Mello NM, Bourne T, et al. Pregnancy of unknown location: A consensus statement of nomenclature, definitions, and outcome. *Fertil Steril*. 2011;95(3):857-866. doi: 10.1016/j.fertnstert.2010.09.006 [doi].

179. Maesawa Y, Yamada H, Deguchi M, Ebina Y. History of biochemical pregnancy was associated with the subsequent reproductive failure among women with recurrent spontaneous abortion. *Gynecol Endocrinol*. 2015;31(4):306-308. doi: 10.3109/09513590.2014.994601 [doi].

180. Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. *Hum Reprod*. 1999;14(11):2868-2871. doi: 10.1093/humrep/14.11.2868 [doi].

181. Lund M, Kamper-Jorgensen M, Nielsen HS, Lidegaard O, Andersen AM, Christiansen OB. Prognosis for live birth in women with recurrent miscarriage: What is the best measure of success? *Obstet Gynecol*. 2012;119(1):37-43. doi: 10.1097/AOG.0b013e31823c0413 [doi].

182. Simon C, Landeras J, Zuzuarregui JL, Martin JC, Remohi J, Pellicer A. Early pregnancy losses in in vitro fertilization and oocyte donation. *Fertil Steril*. 1999;72(6):1061-1065. doi: S0015-0282(99)00408-2 [pii].

183. Bellver J, Albert C, Labarta E, Pellicer A. Early pregnancy loss in women stimulated with gonadotropin-releasing hormone antagonist protocols according to oral contraceptive pill pretreatment. *Fertil Steril*. 2007;87(5):1098-1101. doi: S0015-0282(06)04411-6 [pii].

184. Letter: Where have all the conceptions gone? *Lancet*. 1975;1(7907):636-637. doi: S0140-6736(75)91920-0 [pii].

185. Chard T. Frequency of implantation and early pregnancy loss in natural cycles. *Baillieres Clin Obstet Gynaecol*. 1991;5(1):179-189. doi: 10.1016/s0950-3552(05)80077-x [doi].

186. Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: Population based register linkage study. *BMJ*. 2000;320(7251):1708-1712. doi: 10.1136/bmj.320.7251.1708 [doi].

187. Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. *Hum Reprod*. 1999;14(11):2868-2871. doi: 10.1093/humrep/14.11.2868 [doi].

188. Hassold T, Chiu D. Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. *Hum Genet*. 1985;70(1):11-17. doi: 10.1007/bf00389450 [doi].

189. Li TC, Iqbal T, Anstie B, et al. An analysis of the pattern of pregnancy loss in women with recurrent miscarriage. *Fertil Steril*. 2002;78(5):1100-1106. doi: S0015028202042073 [pii].

190. Clifford K, Rai R, Regan L. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. *Hum Reprod*. 1997;12(2):387-389. doi: 10.1093/humrep/12.2.387 [doi].

191. Tuckerman E, Laird SM, Stewart R, Wells M, Li TC. Markers of endometrial function in women with unexplained recurrent pregnancy loss: A comparison between morphologically normal and retarded endometrium. *Hum Reprod*. 2004;19(1):196-205.

192. Tang AW, Alfirevic Z, Quenby S. Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: A systematic review. *Hum Reprod*. 2011;26(8):1971-1980. doi: 10.1093/humrep/der164 [doi].

193. Saravelos SH, Regan L. The importance of preconception counseling and early pregnancy monitoring. *Semin Reprod Med*. 2011;29(6):557-568. doi: 10.1055/s-0031-1293209 [doi].

194. Rai R, Sacks G, Trew G. Natural killer cells and reproductive failure--theory, practice and prejudice. *Hum Reprod*. 2005;20(5):1123-1126. doi: deh804 [pii].

195. Quenby S, Bates M, Doig T, et al. Pre-implantation endometrial leukocytes in women with recurrent miscarriage. *Hum Reprod*. 1999;14(9):2386-2391. doi: 10.1093/humrep/14.9.2386 [doi].

196. Clifford K, Flanagan AM, Regan L. Endometrial CD56+ natural killer cells in women with recurrent miscarriage: A histomorphometric study. *Hum Reprod*. 1999;14(11):2727-2730. doi: 10.1093/humrep/14.11.2727 [doi].

197. Chard T. Frequency of implantation and early pregnancy loss in natural cycles. *Baillieres Clin Obstet Gynaecol*. 1991;5(1):179-189. doi: 10.1016/s0950-3552(05)80077-x [doi].

198. Ogasawara M, Aoki K, Okada S, Suzumori K. Embryonic karyotype of abortuses in relation to the number of previous miscarriages. *Fertil Steril*. 2000;73(2):300-304. doi: S0015-0282(99)00495-1 [pii].

199. Carp H, Toder V, Aviram A, Daniely M, Mashiach S, Barkai G. Karyotype of the abortus in recurrent miscarriage. *Fertil Steril*. 2001;75(4):678-682. doi: S0015-0282(00)01801-X [pii].

200. Fox CW, Savaris RF, Jeong J, et al. Unexplained recurrent pregnancy loss and unexplained infertility: Twins in disguise. *Human Reproduction Open*. 2020;2020(1):hoz021.

201. Liddell HS, Pattison NS, Zanderigo A. Recurrent miscarriage--outcome after supportive care in early pregnancy. *Aust N Z J Obstet Gynaecol*. 1991;31(4):320-322. doi: 10.1111/j.1479-828x.1991.tb02811.x [doi].

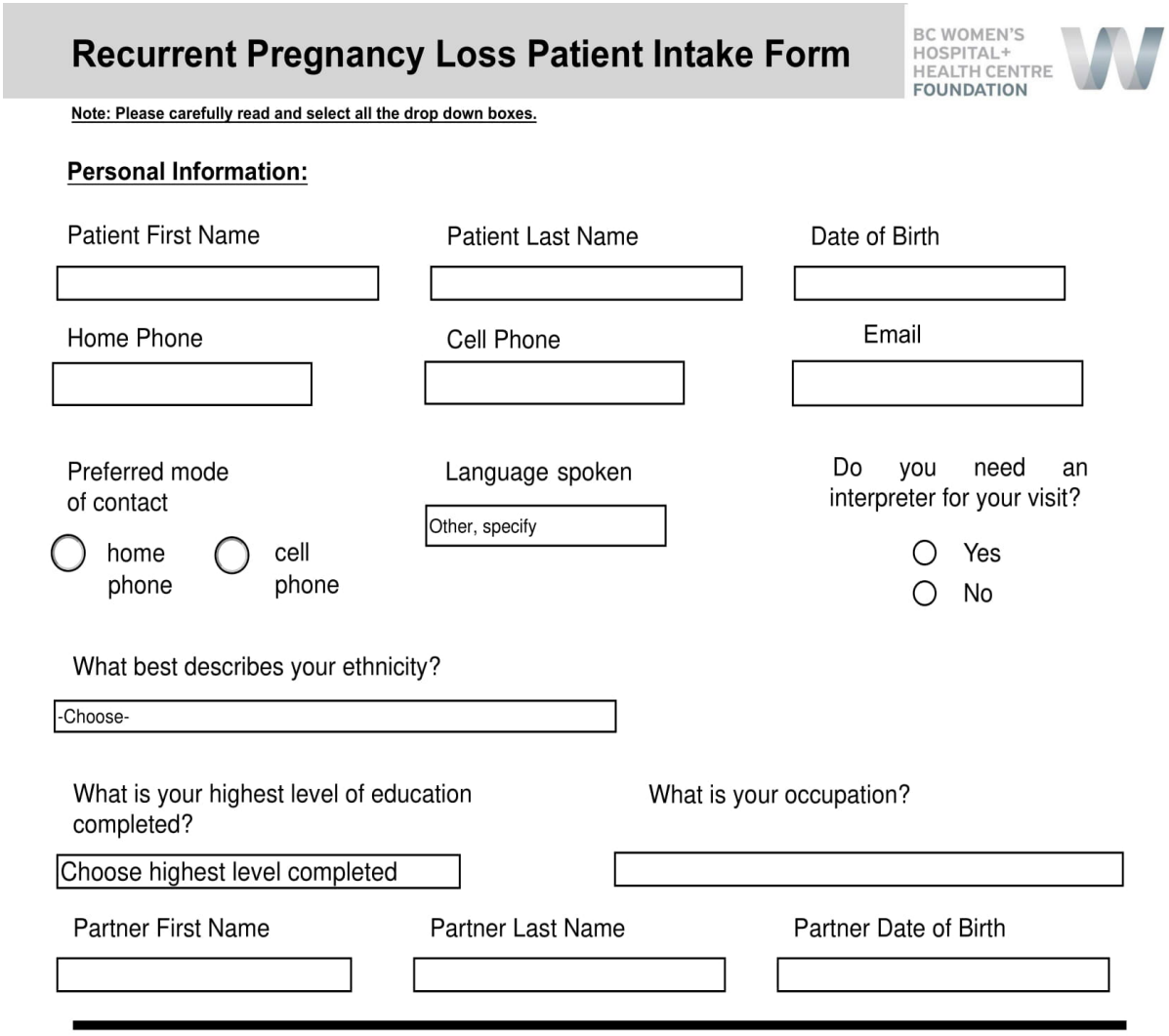
202. Saravelos SH, Li TC. Unexplained recurrent miscarriage: How can we explain it? *Hum Reprod*. 2012;27(7):1882-1886. doi: 10.1093/humrep/des102 [doi].

203. James S, Gil KM, Myers NA, Stewart J. Effect of parity on gestational age at delivery in multiple gestation pregnancies. Journal of Perinatology. 2009;29(1):13-19.

Appendices

Appendix A

A.1 Recurrent pregnancy loss patient intake form



Recurrent Pregnancy Loss Patient Intake Form

BC WOMEN'S HOSPITAL+ HEALTH CENTRE FOUNDATION

Note: Please carefully read and select all the drop down boxes.

Personal Information:

Patient First Name | Patient Last Name | Date of Birth

Home Phone | Cell Phone | Email

Preferred mode of contact: home phone / cell phone

Language spoken: Other, specify

Do you need an interpreter for your visit? Yes / No

What best describes your ethnicity? -Choose-

What is your highest level of education completed? Choose highest level completed

What is your occupation?

Partner First Name | Partner Last Name | Partner Date of Birth

Gynaecological History

BC WOMEN'S HOSPITAL+ HEALTH CENTRE FOUNDATION

Menstrual Cycle Information:

Age of onset of Periods

Periods ◯ Regular ◯ Irregular ◯ Unknown

Average length of cycle:
(Days from first day of period to next period)

Duration of Periods

Date of Last Menstrual Period: (LMP)

Intermenstrual spotting ◯ Yes ◯ No ◯ Unknown

Painful Intercourse (Dyspareunia): ◯ Yes ◯ No ◯ Unknown

Painful periods with cramps: (Dysmenorrhea) ◯ Yes ◯ No ◯ Unknown

Estimated Flow ◯ Light ◯ Moderate ◯ Heavy ◯ Unknown

Aware of ovulation ◯ Yes ◯ No ◯ Unknown

Pre Menstrual Symptoms (PMS): ◯ Yes ◯ No ◯ Unknown

Pelvic pain between periods ◯ Yes ◯ No ◯ Unknown

Others:

IUD usage ◯ Yes ◯ No ◯ Unknown

Oral Contraceptive Usage ◯ Yes ◯ No ◯ Unknown

Galactorrhea(milky discharge from breast) ◯ Yes ◯ No ◯ Unknown

Infertility (Unable to get pregnant from last 6 or more than 6 months) ◯ Yes ◯ No ◯ Unknown

History of STD(Sexually Transmitted Diseases, Pelvic infection) ◯ Yes ◯ No ◯ Unknown

Endometriosis ◯ Yes ◯ No ◯ Unknown

Pap Smears

◯ Normal Date of Last Pap dd-mm-yyy

◯ Abnormal ever Date of Last Pap dd-mm-yyy

Please check the one that applies:

☐ Colposcopy ☐ Conization

☐ LEEP ☐ Cryotherepy

◯ No

◯ Unknown

Medical History

BC WOMEN'S HOSPITAL+ HEALTH CENTRE FOUNDATION

Have you had any of the following?

- ☐ Drug allergies
- ☐ Other allergies
- ☐ High blood pressure
- ☐ Heart disease (angina, abnormal heart rhythm)
- ☐ High or low thyroid
- ☐ Diabetes
- ☐ Neurologic (migraines, seizures)
- ☐ Autoimmune (Lupus,Rheumatoid Arthritis, Raynaud.Ulqitgp)u+
- ☐ Kphrcoocvqt{"dqygn"fkugcug*Etqjp)u.Whegtcvkxg"eqnkvku+
- ☐ Vjtqodqgodqnkuo *blood clots in legs or Lungs+
- ☐ Cpgokc*Ktqp"fghkekgpe{.vjcncuugokc.ukemgn"egm"cpgokc+
- ☐ Asthma
- ☐ Kidney disease or Urinary tract Infections
- ☐ Liver disease(hepatitis, jaundice)
- ☐ None of the above

Please list any other medical problems?

Are there any specialists that you have seen?

Please specify:

Surgical History

Please specify any surgery you have had in the past.

- ☐ Cervix
- ☐ Ovaries
- ☐ Uterus
- ☐ Others

Medication

Please list any medications that you take regularly (including vitamins and non prescription medications).

Personal History

Do you smoke?

- ◯ Yes
- ◯ No

How much alcohol do you consume in an average week?

Please specify:

Stress:

Please specify

Please check any current or past substance use.

- ☐ Marijuana
- ☐ Cocaine
- ☐ Heroin
- ☐ IV drugs (any)
- ☐ Others

Family History

Do you have a family history if any of the following?

- ☐ High blood pressure
- ☐ Diabetes
- ☐ Blood clots in legs or lungs
- ☐ Autoimmune disease (e.g. Rheumatoid arthritis, Lupus, Raynauds, Sjogren's)
- ☐ Chromosomal (genetic) problems (Down's syndrome, thalassemia/anemia, translocations)
- ☐ Congenital anomalies and birth defects
- ☐ Thyroid disease
- ☐ Arthritis
- ☐ Infertility
- ☐ Recurrent pregnancy loss
- ☐ None of the above
- ☐ Others ☐

Investigations

Please check off any testing that may have had already so that we can get results prior to your visit.

Pelvic ultrasound	◯ Yes	◯ No
Hysterosalpingogram (HSG)	◯ Yes	◯ No
Hysteroscopy	◯ Yes	◯ No
Endometrial Biopsy	◯ Yes	◯ No
Chromosome (genetic) testing of you and your partner	◯ Yes	◯ No
Hormonal blood tests"(thyroid, FSH."F|cdg|gu)	◯ Yes	◯ No
Blood tests for blood clotting problems (lupus anticoagulant, anticardiolipin, Beta 2 glycoprotein)	◯ Yes	◯ No
Laparoscopy	◯ Yes	◯ No
Laparotomy	◯ Yes	◯ No

Others: ☐

PARTNER HISTORY

BC WOMEN'S HOSPITAL+ HEALTH CENTRE FOUNDATION

Partner number:

Personal Infromation

Partner's name:

Partner's DOB:

Partner's age:

Year relationship began

Language spoken:

Ethnicity: What best describes your ethnicity?

Occupation

Sex: ◯ F ◯ M

PARTNER MEDICAL HISTORY

Have you had any of the following?

- ☐ Drug allergies
- ☐ Other allergies
- ☐ Diabetes
- ☐ Thyroid disease
- ☐ Arthritis
- ☐ Cardiac disease
- ☐ Urinary tract infections
- ☐ History of blood transfusions
- ☐ Past surgeries
- ☐ Infertility
- ☐ Previous Chemotherapy
- ☐ Other
- ☐ None

PARTNER PERSONAL HISTORY

BC WOMEN'S HOSPITAL+ HEALTH CENTRE FOUNDATION

Do you smoke?

- ○ Yes
- ○ No

How much alcohol do you consume in an average week?

Please specify:

Please check any current or past substance use:

- ☐ Marijuana
- ☐ Cocaine
- ☐ Heroin
- ☐ IV drugs (any)
- ☐ Others

Stress:

Please specify

Family History:

- ☐ High blood pressure
- ☐ Diabetes
- ☐ Blood clots in legs and lungs
- ☐ Autoimmune disease (e.g. Rheumatoid arthritis, Lupus, Raynauds, Sjogren's)
- ☐ Chromosomal (genetic) problems (Down's syndrome, thalassemia/anemia, translocations)
- ☐ Congenital anomalies and birth defects
- ☐ Thyroid disease
- ☐ Arthritis
- ☐ Infertility
- ☐ Recurrent pregnancy loss
- ☐ None of the above
- ☐ Others

Pregnancy History

How many pregnancies have you had?	
How many term deliveries have you had (at or more than 37 weeks)?	
How many preterm deliveries have you had (between 20 weeks and 36+6 weeks)?	
How many miscarriages <20 weeks have you had?	
How many pregnancies of unknown location have you had?	
How many ectopic (tubal) pregnancies have you had?	
How many molar pregnancies have you had?	
How many living children do you have?	

Pregnancy #1

BC WOMEN'S HOSPITAL+ HEALTH CENTRE FOUNDATION

Date of completion of this pregnancy

Age at the time of this pregnancy completion

Partner #

What fertility treatment was used,if any, for this pregnancy -

Gestational Age

Pregnancy duration by menstrual period dates (how many weeks and days were you pregnant at the time this pregnancy ended?)

Pregnancy duration by ultrasound (how many weeks and days were you pregnant by ultrasound, when this pregnancy ended?)

Was Ultrasound done? ◯ Yes ◯ No

What was found by Ultrasound?

Outcome of pregnancy #1

--

If this pregnancy was a first trimester miscarriage how did it end?

-

Record of this miscarriage available?

◯ No ◯ Yes, in what hospital did this take place (hospital name, city)?

If this pregnancy was a second trimester miscarriage, please provide details:

If this pregnancy was preterm or term birth, please provide mode of delivery:

-

Provide more details

Was there chromosomal (genetic) testing of the pregnancy?

Do you know the results of the genetic testing of the pregnancy?

-

Do you have any complications?

-

For office use only

Outcome during the first trimester:

☐ **Non-visualized Pregnancy** ☐ Biochemical PL ☐ Resolved PUL ☐ Treated PUL

☐ **Miscarriage** ☐ ***Early*** ☐ wks ☐ d ☐ Anembryonic ☐ Yolk sac ☐ Embryonic ☐ ***Fetal*** ☐ wks ☐ d

☐ **Ectopic**

☐ **Ongoing Pregnancy**

Pregnancy #2

BC WOMEN'S HOSPITAL+ HEALTH CENTRE FOUNDATION

Date of completion of this pregnancy

Age at the time of this pregnancy completion

Partner #

What fertility treatment was used,if any, for this pregnancy -

Gestational Age

Pregnancy duration by menstrual period dates (how many weeks and days were you pregnant at the time this pregnancy ended?)

Pregnancy duration by ultrasound (how many weeks and days were you pregnant by ultrasound, when this pregnancy ended?)

Was Ultrasound done? ◯ Yes ◯ No

What was found by Ultrasound?

Outcome of pregnancy #2

-

If this pregnancy was a first trimester miscarriage how did it end?

-

Record of this miscarriage available?

◯ No ◯ Yes, in what hospital did this take place (hospital name, city)?

If this pregnancy was a second trimester miscarriage, please provide details:

If this pregnancy was preterm or term birth, please provide mode of delivery:

-

Provide more details

Was there chromosomal (genetic) testing of the pregnancy?

Do you know the results of the genetic testing of the pregnancy?

-

Do you have any complications?

For office use only

Outcome during the first trimester:

☐ **Non-visualized Pregnancy** ☐ Biochemical PL ☐ Resolved PUL ☐ Treated PUL

☐ **Miscarriage** ☐ ***Early*** ☐ wks ☐ d ☐ Anembryonic ☐ Yolk sac ☐ Embryonic ☐ ***Fetal*** ☐ wks ☐ d

☐ **Ectopic**

☐ **Ongoing Pregnancy**

Pregnancy #3

BC WOMEN'S HOSPITAL+ HEALTH CENTRE FOUNDATION

Date of completion of this pregnancy

Age at the time of this pregnancy completion

Partner #

What fertility treatment was used,if any, for this pregnancy -

Gestational Age

Pregnancy duration by menstrual period dates (how many weeks and days were you pregnant at the time this pregnancy ended?)

Pregnancy duration by ultrasound (how many weeks and days were you pregnant by ultrasound, when this pregnancy ended?)

Was Ultrasound done? ◯ Yes ◯ No

What was found by Ultrasound?

Outcome of pregnancy #3

-

If this pregnancy was a first trimester miscarriage how did it end?

-

Record of this miscarriage available?

◯ No ◯ Yes, in what hospital did this take place (hospital name, city)?

If this pregnancy was a second trimester miscarriage, please provide details:

If this pregnancy was preterm or term birth, please provide mode of delivery:

-

Provide more details

Was there chromosomal (genetic) testing of the pregnancy?

-

Do you know the results of the genetic testing of the pregnancy?

-

Do you have any complications?

-

For office use only

Outcome during the first trimester:

☐ **Non-visualized Pregnancy** ☐ Biochemical PL ☐ Resolved PUL ☐ Treated PUL

☐ **Miscarriage** ☐ ***Early*** ☐ wks ☐ d ☐ Anembryonic ☐ Yolk sac ☐ Embryonic ☐ ***Fetal*** ☐ wks ☐ d

☐ **Ectopic**

☐ **Ongoing Pregnancy**

Pregnancy #4

Date of completion of this pregnancy

Age at the time of this pregnancy completion

Partner #

What fertility treatment was used,if any, for this pregnancy -

Gestational Age

Pregnancy duration by menstrual period dates (how many weeks and days were you pregnant at the time this pregnancy ended?)

Pregnancy duration by ultrasound (how many weeks and days were you pregnant by ultrasound, when this pregnancy ended?)

Was Ultrasound done? ◯ Yes ◯ No

What was found by Ultrasound?

Outcome of pregnancy #4

-

If this pregnancy was a first trimester miscarriage how did it end?

-

Record of this miscarriage available?

◯ No ◯ Yes, in what hospital did this take place (hospital name, city)?

If this pregnancy was a second trimester miscarriage, please provide details:

If this pregnancy was preterm or term birth, please provide mode of delivery:

-

Provide more details

Was there chromosomal (genetic) testing of the pregnancy?

Do you know the results of the genetic testing of the pregnancy?

-

Do you have any complications?

-

For office use only

Outcome during the first trimester:

☐ **Non-visualized Pregnancy** ☐ Biochemical PL ☐ Resolved PUL ☐ Treated PUL

☐ **Miscarriage** ☐ ***Early*** ☐ wks ☐ d ☐ Anembryonic ☐ Yolk sac ☐ Embryonic ☐ ***Fetal*** ☐ wks ☐ d

☐ **Ectopic**

☐ **Ongoing Pregnancy**

Pregnancy #5

BC WOMEN'S HOSPITAL+ HEALTH CENTRE FOUNDATION

Date of completion of this pregnancy

Age at the time of this pregnancy completion

Partner #

What fertility treatment was used,if any, for this pregnancy -

Gestational Age

Pregnancy duration by menstrual period dates (how many weeks and days were you pregnant at the time this pregnancy ended?)

Pregnancy duration by ultrasound (how many weeks and days were you pregnant by ultrasound, when this pregnancy ended?)

Was Ultrasound done? ◯ Yes ◯ No

What was found by Ultrasound?

Outcome of pregnancy #5

-

If this pregnancy was a first trimester miscarriage how did it end?

-

Record of this miscarriage available?

◯ No ◯ Yes, in what hospital did this take place (hospital name, city)?

If this pregnancy was a second trimester miscarriage, please provide details:

If this pregnancy was preterm or term birth, please provide mode of delivery:

-

Provide more details

Was there chromosomal (genetic) testing of the pregnancy?

-

Do you know the results of the genetic testing of the pregnancy?

-

Do you have any complications?

-

For office use only

Outcome during the first trimester:

☐ **Non-visualized Pregnancy** ☐ Biochemical PL ☐ Resolved PUL ☐ Treated PUL

☐ **Miscarriage** ☐ ***Early*** ☐ wks ☐ d ☐ Anembryonic ☐ Yolk sac ☐ Embryonic ☐ ***Fetal*** ☐ wks ☐ d

☐ **Ectopic**

☐ **Ongoing Pregnancy**

Pregnancy #6

Date of completion of this pregnancy

Age at the time of this pregnancy completion

Partner #

What fertility treatment was used,if any, for this pregnancy -

Gestational Age

Pregnancy duration by menstrual period dates (how many weeks and days were you pregnant at the time this pregnancy ended?)

Pregnancy duration by ultrasound (how many weeks and days were you pregnant by ultrasound, when this pregnancy ended?)

Was Ultrasound done? ◯ Yes ◯ No

What was found by Ultrasound?

Outcome of pregnancy #6

-

If this pregnancy was a first trimester miscarriage how did it end?

-

Record of this miscarriage available?

◯ No ◯ Yes, in what hospital did this take place (hospital name, city)?

If this pregnancy was a second trimester miscarriage, please provide details:

If this pregnancy was preterm or term birth, please provide mode of delivery:

-

Provide more details

Was there chromosomal (genetic) testing of the pregnancy?

-

Do you know the results of the genetic testing of the pregnancy?

-

Do you have any complications?

-

For office use only

Outcome during the first trimester:

☐ **Non-visualized Pregnancy** ☐ Biochemical PL ☐ Resolved PUL ☐ Treated PUL

☐ **Miscarriage** ☐ ***Early*** ☐ wks ☐ d ☐ Anembryonic ☐ Yolk sac ☐ Embryonic ☐ ***Fetal*** ☐ wks ☐ d

☐ **Ectopic**

☐ **Ongoing Pregnancy**

Pregnancy #7

BC WOMEN'S HOSPITAL+ HEALTH CENTRE FOUNDATION

Date of completion of this pregnancy

Age at the time of this pregnancy completion

Partner #

What fertility treatment was used,if any, for this pregnancy -

Gestational Age

Pregnancy duration by menstrual period dates (how many weeks and days were you pregnant at the time this pregnancy ended?)

Pregnancy duration by ultrasound (how many weeks and days were you pregnant by ultrasound, when this pregnancy ended?)

Was Ultrasound done? ◯ Yes ◯ No

What was found by Ultrasound?

Outcome of pregnancy #7

-

If this pregnancy was a first trimester miscarriage how did it end?

-

Record of this miscarriage available?

◯ No ◯ Yes, in what hospital did this take place (hospital name, city)?

If this pregnancy was a second trimester miscarriage, please provide details:

If this pregnancy was preterm or term birth, please provide mode of delivery:

-

Provide more details

Was there chromosomal (genetic) testing of the pregnancy?

-

Do you know the results of the genetic testing of the pregnancy?

-

Do you have any complications?

-

For office use only

Outcome during the first trimester:

☐ **Non-visualized Pregnancy** ☐ Biochemical PL ☐ Resolved PUL ☐ Treated PUL

☐ **Miscarriage** ☐ ***Early*** ☐ wks ☐ d ☐ Anembryonic ☐ Yolk sac ☐ Embryonic ☐ ***Fetal*** ☐ wks ☐ d

☐ **Ectopic**

☐ **Ongoing Pregnancy**

Pregnancy #8

Date of completion of this pregnancy

Age at the time of this pregnancy completion

Partner #

What fertility treatment was used,if any, for this pregnancy -

Gestational Age

Pregnancy duration by menstrual period dates (how many weeks and days were you pregnant at the time this pregnancy ended?)

Pregnancy duration by ultrasound (how many weeks and days were you pregnant by ultrasound, when this pregnancy ended?)

Was Ultrasound done? ◯ Yes ◯ No

What was found by Ultrasound?

Outcome of pregnancy #8

-

If this pregnancy was a first trimester miscarriage how did it end?

-

Record of this miscarriage available?

◯ No ◯ Yes, in what hospital did this take place (hospital name, city)?

If this pregnancy was a second trimester miscarriage, please provide details:

If this pregnancy was preterm or term birth, please provide mode of delivery:

-

Provide more details

Was there chromosomal (genetic) testing of the pregnancy?

-

Do you know the results of the genetic testing of the pregnancy?

-

Do you have any complications?

-

For office use only

Outcome during the first trimester:

☐ **Non-visualized Pregnancy** ☐ Biochemical PL ☐ Resolved PUL ☐ Treated PUL

☐ **Miscarriage** ☐ ***Early*** ☐ wks ☐ d ☐ Anembryonic ☐ Yolk sac ☐ Embryonic ☐ ***Fetal*** ☐ wks ☐ d

☐ **Ectopic**

☐ **Ongoing Pregnancy**

Pregnancy #9

Date of completion of this pregnancy

Age at the time of this pregnancy completion

Partner #

What fertility treatment was used,if any, for this pregnancy -

Gestational Age

Pregnancy duration by menstrual period dates (how many weeks and days were you pregnant at the time this pregnancy ended?)

Pregnancy duration by ultrasound (how many weeks and days were you pregnant by ultrasound, when this pregnancy ended?)

Was Ultrasound done? ◯ Yes ◯ No

What was found by Ultrasound?

Outcome of pregnancy #9

-

If this pregnancy was a first trimester miscarriage how did it end?

-

Record of this miscarriage available?

◯ No ◯ Yes, in what hospital did this take place (hospital name, city)?

If this pregnancy was a second trimester miscarriage, please provide details:

If this pregnancy was preterm or term birth, please provide mode of delivery:

-

Provide more details

Was there chromosomal (genetic) testing of the pregnancy?

-

Do you know the results of the genetic testing of the pregnancy?

-

Do you have any complications?

-

For office use only

Outcome during the first trimester:

☐ **Non-visualized Pregnancy** ☐ Biochemical PL ☐ Resolved PUL ☐ Treated PUL

☐ **Miscarriage** ☐ ***Early*** ☐ wks ☐ d ☐ Anembryonic ☐ Yolk sac ☐ Embryonic ☐ ***Fetal*** ☐ wks ☐ d

☐ **Ectopic**

☐ **Ongoing Pregnancy**

Pregnancy #10

Date of completion of this pregnancy

Age at the time of this pregnancy completion

Partner #

What fertility treatment was used,if any, for this pregnancy -

Gestational Age

Pregnancy duration by menstrual period dates (how many weeks and days were you pregnant at the time this pregnancy ended?)

Pregnancy duration by ultrasound (how many weeks and days were you pregnant by ultrasound, when this pregnancy ended?)

Was Ultrasound done? ◯ Yes ◯ No

What was found by Ultrasound?

Outcome of pregnancy #10

-

If this pregnancy was a first trimester miscarriage how did it end?

-

Record of this miscarriage available?

◯ No ◯ Yes, in what hospital did this take place (hospital name, city)?

If this pregnancy was a second trimester miscarriage, please provide details:

If this pregnancy was preterm or term birth, please provide mode of delivery:

-

Provide more details

Was there chromosomal (genetic) testing of the pregnancy?

-

Do you know the results of the genetic testing of the pregnancy?

-

Do you have any complications?

-

For office use only

Outcome during the first trimester:

☐ **Non-visualized Pregnancy** ☐ Biochemical PL ☐ Resolved PUL ☐ Treated PUL

☐ **Miscarriage** ☐ ***Early*** ☐ wks ☐ d ☐ Anembryonic ☐ Yolk sac ☐ Embryonic ☐ ***Fetal*** ☐ wks ☐ d

☐ **Ectopic**

☐ **Ongoing Pregnancy**

Appendix B

B.1 Table showing full clinical workup ordered at RPL initial visit.

Factors	Investigations	Recommendation
Hormonal	TSH, anti-TPO	All patients
	HbA1C	All patients
	Prolactin	If patients report menstrual cycles ≥38 days, blurry vision/headaches, or irregular menstrual cycles
Autoimmune	Lupus Anticoagulant	History of ≥1 pregnancy loss between 10-20 weeks OR History of ≥3 unexplained, consecutive, spontaneous pregnancy losses at <10 weeks of gestation OR History of ≥1 preterm delivery of a normal infant < 34 weeks due to severe preeclampsia, eclampsia, or placental insufficiency
	Anticardiolipin Ab (IgM/IgG)	
	Beta 2 Glycoprotein Ab (IgM/IgG)	
Genetic	Parental karyotype	History of ≥ 3 pregnancy losses which are aneuploid (not 46XX/46XY) OR History of any conceptus with deletion/duplication of chromosomes seen in a prior loss OR Family history of ≥2 losses in the parents, sibling of either partner
Anatomic	HSG or hysteroscopy or sonohysterogram	All patients HSG can be done prior to the initial visit in the community
Thrombophilia	Factor V	History of Deep vein thrombosis/Pulmonary embolism at ≥ 1 occasion OR Family history of 1st-degree relative with thrombophilia OR History of pregnancy loss at 10-20 gestational week associated with thrombosis in the surgical path (nor abruption)
	Antithrombin	
	Prothrombin gene mutation	
Serologies	Rubella	All patients
	Varicella	
	RPR	
	HIV	
	HepBsAg	
	Anti-HCV	

Appendix C

C.1 Table showing the steps of pregnancy monitoring at BCWH clinic.

Step 1: Referral	Accepted: If patient has not previously had a term pregnancy through our pregnancy monitoring program.
Step 2: Recurrent Pregnancy Monitoring Intake form	Completed by a Registered Nurse
Step 3: Order Lab work	
Compulsory for all patients	HCG measured two times 48 hours apart
	Blood group/Rhesus
	Rubella, Varicella, RPR, HIV, Hepatitis B, Hepatitis C
Patients with History of positive TPO antibody *or* On levothyroxine *or* Prior TSH ≥ 2.5	TSH
+	
Patients working with children	CMV, Toxoplasmosis, Parvo serology
+	
Patients taking Dalteparin	CBC (to check platelets) and 2 weeks later
Step 4: Booking the first visit	Registered nurse books the initial visit after reviewing the test results.
Case of known hypothyroidism (TSH< 2.5)	Patient advised to take a double dose of Levothyroxine on two days of the week
Ultrasound History of Ectopic pregnancy An inappropriate rise in hCG Everyone else	Initial ultrasound and clinician appointment 5 to 6 weeks 5 to 6 weeks 6 to 7 weeks