Recurrent Pregnancy Loss

**POLICY**

*Please see amendment for Pennsylvania Medicaid at the end of this CPB.*

I. Aetna considers the following tests medically necessary for evaluation of members with recurrent pregnancy loss (defined as 2 or more consecutive spontaneous abortions):

A. Endometrial biopsies for evaluation of luteal phase defect;

B. Factor V Leiden genetic testing for members with an abnormal activated protein C (APC) resistance assay result (see CPB 0140 - Genetic Testing ([../100_199/0140.html])

C. Hysterosalpingography, hysteroscopy or sonohysteroscopy/sonohysterography to diagnose uterine anatomic abnormalities;

D. Karyotype (cytogenetic analysis) of parents to detect balanced chromosomal anomalies;

E. Karyotype of abortus tissue when a couple with recurrent pregnancy loss experiences a subsequent spontaneous abortion;

F. Measurement of anti-beta2-glycoprotein I (IgG or IgM) antibodies, anti-cardiolipin (IgG or IgM) antibodies, and lupus anticoagulant, using standard assays, for diagnosis of antiphospholipid syndrome;

G. Pelvic ultrasound scan to assess ovarian morphology and the uterine cavity;

H. Prenatal genetic diagnosis for all couples in which 1 partner has been found to have a balanced translocation or inversion;

I. Tests for thyroid stimulating hormone (TSH) and thyroid antibodies.
II. Aetna considers any of the following tests/studies experimental and investigational because they have been shown to be of no value in the evaluation of recurrent pregnancy loss:

A. Annexin A5 promoter haplotype M2 testing;
B. Angiotensin converting enzyme (ACE) gene polymorphisms testing;
C. Antibodies to phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid or other anti-phospholipid antibodies other than anti-cardiolipin and lupus anticoagulant;
D. Antidiadrenal antibodies;
E. Antinuclear antibody (ANA),
F. Antiovarian antibodies;
G. Cytokine polymorphisms analysis (Th1/Th2 intra-cellular cytokine ratio);
H. Determination of the percentage of circulating natural killer (NK) cells and NK activity;
I. Embryo toxicity assay (ETA) or embryo toxic factor;
J. Estrogen receptor beta gene polymorphisms testing;
K. Expression of peroxisome proliferator activation receptors (PPARs) and tumor necrosis factor alpha (TNFα) in placenta tissues;
L. Expression of pinopodes, thrombomodulin and ezrin in the endometrium;
M. Genetic association studies of inflammatory cytokine polymorphisms;
N. Inhibin B;
O. Interleukin genes polymorphisms testing (including IL-1β, IL-6, IL-10, IL-17, IL-18, IL-21);
P. Inter-α trypsin inhibitor-heavy chain 4 (ITI-H4) (as a biomarker for recurrent pregnancy loss);
Q. Luteal phase biopsy to determine the status of natural killer (NK)-like cells;
R. Maternal antiparental antibodies;
S. Maternal antileukocytic antibodies to paternal leukocytes;
T. Methylene tetrahydrofolate reductase (MTHFR) testing;
U. Mitochondrial DNA variations analysis;
V. Mixed lymphocytotoxic antibody tests;
W. Mixed lymphocyte culture reactions;
X. Molecular cytogenetic testing using comparative genomic hybridization (CGH) for chromosomal analysis (e.g., parental blood and products of conception);
Y. Molecular genetic testing for highly skewed X-inactivation patterns;
Z. Ovarian reserve tests (e.g., antral follicle count, and serum anti-Mullerian hormone levels);
AA. Parental human leukocyte antigen (HLA) status;
AB. Placenta-specific protein1 (PLAC1);
AC. Placental expression of pentraxin 3 (PTX3);
AD. Plasminogen activator inhibitor-1 (PAI-1) gene polymorphisms testing;
AE. Plasminogen activator inhibitor-1 (PAI-1) antigen;
AF. Plasminogen activator inhibitor-1 activity;
AG. Preimplantation genetic testing for aneuploidy (PGT-A) (formerly called preimplantation genetic screening (PGS));
AH. Products of Conception (POC) Test (Igenomix);
Al. Prolactin receptor gene polymorphism testing;
Aj. Reproductive immunophenotype (CD3+, CD4+, CD5+, CD8+, CD16+, CD19+, CD56+);
AK. Routine preimplantation embryo aneuploidy screening;
AL. Serum anti-heat shock protein antibodies (e.g., anti-HSP60 and anti-HSP70) levels;
AM. Serum “blocking factor“;
AN. Sperm DNA fragmentation;
AO. Tumor necrosis factor alpha gene polymorphisms testing;
AP. Tests for inherited thrombophilic disorders: antithrombin III antibody; antithrombin III antigen; factor V Leiden (genetic testing); prothrombin G20210A mutation, serum homocysteine, protein C activity, protein C antigen, protein S activity, protein S antigen, prothrombin (Factor II) mutation, and deficiencies of the anticoagulants protein C, protein S, and antithrombin II;
AQ. X-chromosome inactivation study;
AR. Whole exome sequencing for evaluation of a fetus during pregnancy or a terminated fetus (See CPB 0140 - Genetic Testing (../100_199/0140.html))

III. Aetna considers any of the following treatments experimental and investigational for recurrent pregnancy loss because they have not
been shown to be effective for that indication:

A. Donor leukocyte infusion;
B. Immunotherapy with tumor necrosis factor alpha inhibitors;
C. Intravenous immunoglobulin (IVIG) therapy;
D. In-vitro fertilization-preimplantation genetic diagnosis (IVF-PGD);
E. Leukocyte immunization (immunizing the female partner with the male partner's leukocytes);
F. Low-molecular-weight heparin (however, LMWH may be medically necessary in pregnant women with thrombophilic disorders, and for treatment in pregnant women with venous thrombo-embolism - see CPB 0346 - Low-Molecular-Weight Heparins and Thrombin Inhibitors (0346.html));
G. Trophoblast membrane infusion.

BACKGROUND

This policy is based on the recommendations of the American College of Obstetricians and Gynecologists (ACOG, 2001) and the Royal College of Obstetricians and Gynaecologists (RCOG, 2001).

The ACOG guideline *Management of Recurrent Early Pregnancy Loss* reached the following conclusions: "Women with recurrent pregnancy loss should be tested for lupus anticoagulant and anticardiolipin antibodies using standard assays. If test results are positive for the same antibody on two consecutive occasions 6-8 weeks apart, the patients should be treated with heparin and low-dose aspirin during her next pregnancy attempt. Mononuclear cell (leukocyte) immunization and intravenous immunoglobulin (IVIG) are not effective in preventing recurrent pregnancy loss" (ACOG, 2001).

An association between the luteal phase defect and recurrent pregnancy loss is controversial. If a diagnosis of luteal phase defect is sought in a woman with recurrent pregnancy loss, it should be confirmed by endometrial biopsy. Luteal phase support with progesterone is of unproven efficacy.

Couples with recurrent pregnancy loss should be tested for parenteral balanced chromosome abnormalities. Women with recurrent pregnancy
loss and a uterine septum should undergo hysteroscopic evaluation and resection. Cultures for bacteria and viruses and tests for glucose tolerance, thyroid abnormalities, antibodies to infectious agents, anti-nuclear antibodies, anti-thyroid antibodies, paternal human leukocyte antigen status, or maternal anti-parental antibodies are not beneficial and, therefore, are not recommended in the evaluation of otherwise normal women with recurrent pregnancy loss. Couples with otherwise unexplained recurrent pregnancy loss should be counseled regarding the potential for successful pregnancy without treatment.

The Royal College of Obstetricians and Gynaecologists (RCOG) Guidelines on Management of Recurrent Miscarriage (2001) are consistent with ACOG Guidelines. The RCOG recommends the following work-up for recurrent pregnancy loss:

- A pelvic ultrasound scan to assess ovarian morphology and the uterine cavity
- Karyotyping of all fetal products
- Peripheral blood karyotyping in both partners
- Screening tests for anti-phospholipid antibodies (both the lupus anticoagulant and anti-cardiolipin antibodies) performed on 2 separate occasions at least 6 weeks apart. Discordant results should prompt the performance of a 3rd test.

The RCOG guidelines conclude that "the place of all other investigations including a search for newly described thrombophilic defects is unproven and such tests should only be performed in the context of research studies."

The American College of Obstetricians and Gynecologists (2001) state that tests for thrombophilia are not required as part of the evaluation of recurrent pregnancy loss, but may be considered in cases of otherwise unexplained fetal death in the 2nd or 3rd trimesters. "The role of thrombophilia in recurrent pregnancy loss is a controversial subject of current research interests. Tests for factor V Leiden, the prothrombin G20210A mutations, or deficiencies of protein C, protein S, or antithrombin III should be considered in cases of otherwise unexplained fetal death in the second or third trimesters. However, the role of these heritable thrombophilia in recurrent early pregnancy loss is uncertain at present, and tests for these thrombophilia are not required as part of the
evaluation. Whether antithrombotic treatment improves subsequent pregnancy outcomes in women with evidence of thrombophilia is uncertain."

Updated guidelines from the American College of Obstetricians and Gynecologists (2013) state that testing for inherited thrombophilia in women who have experienced recurrent fetal loss is not recommended because it is unclear if anticoagulation therapy reduces recurrence. Although there may be an association in these cases, there is insufficient clinical evidence that antepartum prophylaxis with unfractionated heparin or low molecular weight heparin (LMWH) prevents recurrence in these patients.

Investigators have also found evidence of significantly higher serum homocysteine levels among women with a history of recurrent miscarriage (Krabbeindam et al, 2005; Hague, 2003). Routine folate supplementation is recommended during pregnancy to prevent neural tube defects (USPSTF, 2006). This supplementation should also reduce serum concentrations of homocysteine that may be associated with recurrent pregnancy loss.

A systematic evidence review found insufficient evidence for plasminogen activator inhibitor 4G/5G polymorphism testing in recurrent miscarriage (Augustovski et al, 2006).

The RCOG recommends that in women with recurrent miscarriage who have undergone the above investigations should undergo the following management:

- That all future treatment options are evaluated in randomized controlled trials;
- That treatments of unproven benefit should be abandoned;
- That women with persistently positive tests for anti-phospholipid antibodies are offered treatment with low dose aspirin together with low dose heparin during pregnancy (also the subject of ongoing research);
- Those with karyotypic abnormalities should be seen by a clinical geneticist.

Recurrent spontaneous abortion (habitual abortion or miscarriage) is
defined as at least 2 or 3 spontaneous abortions prior to 20 weeks gestational age with the same partner. Two types of immunotherapy have been explored: (i) injections of paternal leukocytes (paternal white blood cell immunization or paternal cell alloimmunization) and (ii) the use of IVIG.

A meta-analysis of randomized controlled trials of immunotherapy for recurrent miscarriage concluded that IVIG and paternal leukocyte injections provided no significant beneficial effect over placebo in preventing further miscarriages (Porter et al., 2006). The investigators also found no significant benefit for other immunological treatments that have been used for recurrent miscarriage: third party donor cell immunization and trophoblast membrane infusion. The authors of the meta-analysis concluded that it is questionable whether paternal leukocyte injection is an effective treatment for recurrent miscarriage. They also concluded that third party donor leukocytes, trophoblast membranes, and IVIGs appear to provide no significant beneficial effect over placebo in preventing further miscarriages.

An American Society for Reproductive Medicine (2002) Committee Opinion concluded: "IVIG as a treatment for recurrent pregnancy loss should be evaluated in patients who are informed, consenting participants in an institutional review board approved randomized clinical trial. For the management of recurrent spontaneous pregnancy loss IVIG is an experimental treatment."

The University Health Consortium (1999) guidelines on use of immunoglobulin preparations concluded that the use of IVIG for recurrent pregnancy loss is "not recommended."

Embryo toxicity assay (ETA) is a laboratory test performed on a woman who has had recurrent early pregnancy loss. A blood sample from the woman is used to furnish a culture medium for growing mouse embryos. The culture is then examined under microscopy to determine if there are any circulating factors in the blood specimen that are toxic to the developing mouse embryos. There is a lack of adequate evidence in the peer-reviewed published medical literature on the effectiveness of this test in improving clinical outcomes.
Medicine (2004) concluded that the use of IVIG for the management of recurrent spontaneous pregnancy loss is an experimental treatment.

Stephenson and colleagues (2010) conducted a multi-centered, randomized, double-blinded, placebo-controlled trial comparing IVIG with saline in women with idiopathic secondary recurrent miscarriage, defined as a history of at least 1 prior ongoing pregnancy followed by 3 or more consecutive unexplained miscarriages. Subjects received either IVIG 500 mg/kg body weight or the equivalent volume of normal saline. Pre-conception infusions were administered 14 to 21 days from the projected next menstrual period. With documentation of pregnancy, the subject received the same infusion every 4 weeks until 18 to 20 weeks of gestation. The primary outcome was an ongoing pregnancy of at least 20 weeks of gestation. A total of 82 patients enrolled, of whom 47 had an index pregnancy. All ongoing pregnancies resulted in live births. Thus, the live birth rates were 70% (16/23) in the IVIG group and 63% (15/24) in the control group (p = 0.760); odds ratio (OR) 1.37 [95% confidence interval (CI): 0.41 to 4.61]. Including only clinical pregnancies (embryo with cardiac activity at 6 weeks of gestation), the live birth rates were equivalent, 94% (16/17) and 94% (15/16), respectively (p > 0.999); OR 1.07 (95% CI: 0.06 to 18.62). Meta-analysis of randomized controlled trials (RCTs) evaluating IVIG for idiopathic secondary recurrent miscarriage revealed live birth rates of 70% (31/44) in the IVIG group and 62% (28/45) in the control group (p = 0.503); common OR 1.44 (95% CI: 0.59 to 3.48). The authors concluded that this is the largest RCT to date in which IVIG was evaluated in women with idiopathic secondary recurrent miscarriage; no treatment benefit was found. The meta-analysis, which combined these results with 2 prior RCTs, also showed no significant effect of treatment with IVIG.

In a review on genetics for recurrent pregnancy loss, Sierra and Stephenson (2006) stated that recent research has generated interest in genetic markers for recurrent pregnancy loss such as skewed X-chromosome inactivation and human leukocyte antigen-G polymorphisms. Assisted reproductive technologies (specifically, pre-implantation genetic diagnosis) have been offered to couples with recurrent pregnancy loss; however, more research is needed before routine use of these new approaches can be advocated.

Stephenson and Kutteh (2007) stated that recurrent pregnancy loss
affects up to 5% of couples trying to establish a family. Evaluation classically begins after 3 consecutive miscarriages of less than 10 weeks of gestation, but may be warranted earlier if a prior miscarriage was found to be euploid, or if there is concomitant infertility and/or advancing maternal age. The evaluation begins with an extensive review of medical history and thorough physical examination, followed by a diagnostic screening protocol. The authors noted that management must be evidence-based; unproven treatments should be avoided.

An American Society for Reproductive Medicine Practice Opinion (ASRM, 2007) concluded that "available evidence does not support the use of PGS [preimplantation genetic screening] as currently performed to improve live-birth rates in patients with recurrent pregnancy loss." This is re-affirmed by the ACOG's Committee Opinion on PGS (2009), which stated that there are no data to support PGS for recurrent unexplained miscarriage and recurrent implantation failures; its use for these indications should be restricted to research studies with appropriate informed consent.

Bombell and McGuire (2008) noted that inflammatory cytokine cascades have been implicated in the pathogenesis of recurrent pregnancy loss (RPL). Polymorphisms in cytokine genes may affect the risk of RPL, but genetic association studies are often limited by small sample sizes. Meta-analysis of all available studies can increase the precision of these estimates. These researchers evaluated and synthesized the available data from association studies of inflammatory cytokine polymorphisms with RPL. A total of 16 reports of genetic association studies of cytokine polymorphisms with RPL were identified. Meta-analyses did not identify any significant associations with tumour necrosis factor (−308A, or −238A), interferon-gamma (+874T), interleukin (IL)-1beta (−511T), IL-6 (−174G), or IL-10 (−1082A, or −819T, or −592A). Significant associations were found with IL-1B (−31T) (2 studies: pooled OR 2.12 (95% CI: 1.04 to 4.33)) and IL-6 (−634G) (1 study: OR 0.22 (95% CI: 0.09 to 0.57)). The authors concluded that available data are inconsistent with more than modest associations between these candidate cytokine polymorphisms and RPL. They stated that data from future association studies may be added to the meta-analyses to obtain more precise estimates of effect sizes.

Choi and Kwak-Kim (2008) reviewed cytokine gene polymorphism studies in women with recurrent spontaneous abortion (RSA) to provide comprehensive understanding and a direction for the future investigation.
A search of PubMed was made to identify the published data between 2001 and 2007 regarding RSA and cytokine gene polymorphisms. Either allele and/or genotype frequencies of the following polymorphisms were reported to be significantly different between women with RSA and controls: IFN-gamma +874A-->T, TA (p = 0.01), AA (p = 0.04); IL-6, -634C-->G CG/GG (p = 0.026); IL-10, -592C-->A CC (p = 0.016); IL-1B -511C (p = 0.035), -31T (p = 0.029); IL-1RA, IL1RN*2 (p = 0.002), and IL1RN*3 (p = 0.002). None of these studies was repeatedly reported by others to be significantly different. Among these, 4 cytokine polymorphisms (IFN-gamma, +874A-->T; IL-1B -511C; IL-1RA, IL1RN*2, IL1RN*3) were refuted by others and rest of them were studied once. The authors concluded that multiple cytokine polymorphisms were reported to be associated with RSA. However, a majority of studies were not confirmed by other investigators or refuted by others. Inconsistent study results might be related to: (i) the production of these cytokines is partly under genetic controls and other factors affect cytokine levels; (ii) ethnic background, environmental factors, and selection criteria for study populations are different; and (iii) the possibilities exist that multiple cytokine gene polymorphisms or other genes in linkage disequilibrium may play a role in RSA.

Laskin and colleagues (2009) compared live birth rates in women with RPL and either autoantibodies or a coagulation abnormality, treated with low molecular weight heparin plus aspirin (LMWH/ASA) or aspirin (ASA) alone, and placed the results in context with other RCTs with similar cohorts. The HepASA Trial was an RCT including patients with a history of RPL and at least 1 of the following: anti-phospholipid antibody (aPL), an inherited thrombophilia, or antinuclear antibody. Treatment groups were stratified by aPL status and history of early versus late pregnancy losses. Patients received either LMWH/ASA or ASA alone. The primary outcome was live birth; secondary outcomes included adverse events and bone loss at the spine and femoral neck. Literature over the past 20 years was reviewed to identify comparable RCT. Over 4 years, a total of 859 women with RPL were screened: 88 (10.2 %) fulfilled inclusion criteria, became pregnant and were randomized to receive either LMWH/ASA or ASA alone. Anti-phospholipid antibody were present in 42 (47.7 %) patients in each group. The trial was stopped after 4 years when an interim analysis showed no difference in live birth rates in the 2 groups, and a lower rate of pregnancy loss in the ASA only group than
expected. In the LMWH/ASA group, 35/45 (77.8%) had a live birth versus 34/43 (79.1%) in the ASA only group (p = 0.71). Neither number of prior losses nor aPL status was correlated with pregnancy outcome. There were no cases of pregnancy related thrombosis in either group. Mean change in bone mineral density did not differ by treatment group at either the lumbar spine (p = 0.57) or femoral neck (p = 0.15).

Randomized controlled trials since 2000 for aPL-positive women with RPL and similar inclusion criteria reported a mean live birth rate of 75% with either LMWH or ASA. The authors concluded that LMWH/ASA did not confer incremental benefit compared to ASA alone for this population. Regardless of treatment regimen, number of prior losses, or aPL positivity, almost 80% of women in the RPL cohort had a successful pregnancy outcome. These findings contribute to a growing body of literature that contests the emerging standard of care comprising LMWH/ASA for this population.

Kim and colleagues (2011) stated that there are several etiological factors associated with immunology, anatomy, endocrinology, genetic, infection, chromosomal abnormalities, and environmental factors contributing to RPL. The aim of this study was to identify RPL associated factors in human blood using proteomics. Since it is difficult to obtain tissues or follicular fluids, these researchers used blood samples from normal and RPL patients to conduct a comparative proteomic study. Three RPL blood samples and 1 cocktailed blood sample from 3 normal women were used. These investigators performed 2-DE and selected spots were analyzed with MALDI-TOF/MS. In the 3 RPL blood samples, 2-DE analysis revealed 549, 563 and 533 spots to be expressed differentially, respectively. Through a comparative analysis between the control and RPL, 21 spots were shown to be differentially expressed. Of these, 5 proteins were confirmed by Western blot analysis. One of these proteins, inter-α trypsin inhibitor-heavy chain 4 (ITI-H4), was weakly expressed at a molecular weight of 120 kDa, but was highly expressed at a modified molecular weight of 36 kDa in RPL patients. These findings suggested that ITI-H4 expression may be used as a biomarker, which could facilitate the development of novel diagnostic and therapeutic tools.

The ACOG's practice bulletin on "anti-phospholipid syndrome" (2011) stated that obstetric indications for anti-phospholipid antibody testing (anti-beta2-glycoprotein I of immunoglobulin G [IgG] and/or immunoglobulin M isotype, anti-cardiolipin antibody, and lupus anti-
coagulant) should be limited to a history of 1 fetal loss or 3 or more recurrent embryonic or fetal losses.

Darmochwal-Kolarz et al (2002) estimated the alterations in the phenotype of lymphocytes of women with unexplained pregnancy failures in comparison with healthy women. A total of 14 women with unexplained habitual miscarriages and 18 healthy, fertile women with the history of successful pregnancies were included in the study. The lymphocytes were isolated from peripheral blood and stained with monoclonal antibodies. The expression of selected surface molecules was estimated using the flow cytometric method. These researchers found that the percentage of T CD4(+) lymphocytes, CD3(-)16/56(+) cells, and T CD8(+11b(-) cells was significantly higher in patients with recurrent pregnancy loss in comparison with healthy women. The percentage of B-1 CD19(+)5(+) lymphocytes was also significantly higher in women with unexplained habitual miscarriages in comparison with healthy women. Furthermore, they found higher expression of CD25 molecule on T CD3(+) and T CD4(+) lymphocytes in the study group, when compared to controls. Moreover, the percentages of B CD19(+) and T suppressor CD8(+11b(+)) lymphocytes were lower in women with pregnancy failures in comparison with the control group. The percentage of T CD3(+) lymphocytes and T CD8(+) cells did not differ in both studied groups. Similarly, the expression of CD25 antigen and HLA-DR molecule on T CD8(+) did not differ in the study group, when compared to controls. The authors concluded that these findings suggested that the immunological alterations may be involved in the etiopathogenesis of unexplained recurrent pregnancy loss. The results of this small study need to be validated by well-designed studies.

Inflammatory cytokine cascades have been implicated in the pathogenesis of recurrent pregnancy loss. Polymorphisms in cytokine genes may affect the risk of recurrent pregnancy loss, but genetic association studies are often limited by small sample sizes. Meta-analysis of all available studies can increase the precision of these estimates. In a meta-analysis, Bombell et al (2008) assessed and synthesized the available data from association studies of inflammatory cytokine polymorphisms with recurrent pregnancy loss. Systematic review and random effects meta-analysis of genetic association studies were performed. A total of 16 reports of genetic association studies of cytokine polymorphisms with recurrent pregnancy loss were identified. Meta-
analyses did not identify any significant associations with tumor necrosis factor (-308A, or -238A), interferon-gamma (+874T), interleukin (IL)-1beta (-511T), IL-6 (-174G), or IL-10 (-1082A, or -819T, or -592A). Significant associations were found with IL-1B (-31T) (2 studies: pooled odds ratio (OR) 2.12 (95% CI: 1.04 to 4.33)) and IL-6 (-634G) (1 study: OR 0.22 (95% CI: 0.09 to 0.57)). The authors concluded that available data are not consistent with more than modest associations between these candidate cytokine polymorphisms and recurrent pregnancy loss. Data from future association studies may be added to the meta-analyses to obtain more precise estimates of effect sizes.

Agrawal et al (2012) determined whether or not interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β) and IL-1 receptor antagonist (IL-1RA) polymorphisms are associated with risk of unexplained recurrent pregnancy loss among North Indian women. This retrospective case-control study examined 200 well-characterized recurrent pregnancy loss cases for IL-1 gene cluster variants, determined by restriction fragment length polymorphism-PCR. The observed allele, genotype and haplotype distributions were compared with those obtained from 300 ethnically-matched negative controls. Invariant distribution of IL-1 gene cluster single-nucleotide polymorphisms was observed among recurrent pregnancy loss cases and controls. Meta-analysis of IL-1β -511, +3953 and IL-1RN 86-bp variable number tandem repeat from the reported literature and this study did not reveal any significant association with the risk of recurrent pregnancy loss. The authors concluded that no significant difference between recurrent pregnancy loss and control groups was observed at the allele, genotype or haplotype levels when tested for association using the dominant, recessive and additive models of inheritance for IL-1 gene cluster variants. The authors noted that this is the first report from India pertaining to IL-1 gene cluster variants’ association with the risk of recurrent pregnancy loss from North India.

One of the most unfortunate complications of incomplete motherhood is recurrent pregnancy loss particularly of unknown etiology. Both genetic and environmental factors may play a role in the maintenance of pregnancy. From a traditional immunological perspective, survival of the semi-allogenic fetus is dependent on suppression of the maternal immune response. However, the function of immune cells changes during pregnancy, no generalized suppression of the maternal immune response has been recorded. Cytokines in the interleukin-1 system (IL-1α, IL-1β and receptor antagonist (IL-1RA)) are produced at the fetal-maternal
interface during early pregnancy and are believed to influence the immune responsiveness of Th1/Th2 cells and has been implicated in the establishment of successful pregnancy. This study examined IL-1 gene cluster variants among well-characterized recurrent pregnancy loss patients and compared them with those of healthy controls. Interleukin-1 gene cluster single-nucleotide polymorphisms were found to be invariably distributed among recurrent pregnancy loss patients and controls. Meta-analysis of results of IL-1β -511, IL-1β +3953 and IL-1RN 86-bp variable number tandem repeat studies in recurrent pregnancy loss, as reported previously, also did not show any effect of these variations on the risk of occurrence of the disease.

Rao et al (2008) determined the frequency of hypothyroidism in women with RPL in first trimester in the Indian population. The study included 163 non-pregnant women with RPL in a gestational age up to less than or equal to 12 weeks verified by a pregnancy test or ultrasonography, and a total of 170 age-matched women with at least 1 successful pregnancy and no history of miscarriages were selected as controls. Levels of thyroid hormones triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) were estimated in non-pregnant women with RPL and controls. Hypothyroidism was found in 7 (4.12 %) women with RPL and 1 in control group. The differences in the levels of serum T3, T4 and TSH between euthyroid and hypothyroid women were found significant in women with RPL in first trimester. The authors concluded that the findings of this study demonstrated that hypothyroidism has a statistically significant relationship with RPL in the first trimester and suggested that diagnosis of hypothyroidism could help couples with RPL to have a successful outcome in subsequent pregnancies.

In a single-center, retrospective study, Jaslow and colleagues (2010) examined if the frequency of abnormal results for evidence-based diagnostic tests differed among women with RPL based on the number of prior losses (n = 2, 3, or more) and determine whether abnormal results for additional investigative diagnostic tests differed in prevalence among women with different numbers of pregnancy losses. A total of 1,020 women who had 2 or more consecutive spontaneous pregnancy losses with the same partner were included in this analysis. Main outcome measures included frequencies of abnormal results for evidence-based diagnostic tests considered definite or probable causes of RPL (karyotyping for parental chromosomal abnormalities; pelvic sonohysterography, hysterosalpingogram, or hysteroscopy for uterine
anomalies; immunological tests for lupus anti-coagulant and anti-cardiolipin antibodies; thrombophilic tests for the factor V Leiden mutation; and blood tests for TSH and fasting blood glucose). These investigators also measured the frequency of abnormal results for 9 additional investigative tests in the same patients (antiphosphatidyl serine antibodies, microbial infection, mid-luteal progesterone, prolactin, functional protein C activity, functional protein S activity, anti-thrombin activity, fasting homocysteine and methylenetetrahydrofolate reductase polymorphisms, and factor II mutation). The prevalence of abnormal results for evidence-based and investigative diagnostic tests did not differ among women with different numbers of pregnancy losses. The authors concluded that evaluation of all couples with 2, 3, or more consecutive miscarriages is recommended.

In a case-control study, Ticconi et al (2011) examined the role of anti-thyroid autoantibodies (ATA) in recurrent miscarriage (RM). A total of 160 women with RM and 100 healthy women were investigated for the presence of serum ATA directed against thyroglobulin (TG-Ab), thyroid peroxidase (TPO-Ab) and TSH receptor (TSHr-Ab), which were determined by either chemiluminescence or radioimmunoassay. Anti-thyroid autoantibodies were detected in 46 (28.75 %) women with RM and in 13 (13 %) women of the control group (p < 0.05). The frequencies for TG-Ab and TPO-Ab were higher in RM than in control women. Among the women of RM group, 91.3 % of ATA+ women were positive also for other autoantibodies. The majority of study women were euthyroid. The authors concluded that anti-thyroid autoantibodies, particularly TG-Ab, are associated with RM and could be an expression of a more general maternal immune system abnormality leading to RM. They stated that ATA could have a role in RM irrespective of thyroid hormone status.

van den Boogaard et al (2011) conducted a systematic review of the literature on the clinical significance of thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy. Relevant studies were identified by searching Medline, EMBASE and the Cochrane Controlled Trials Register. From a total of 14,208 primary selected titles, 43 articles were included for the systematic review and 38 were appropriate for meta-analyses. No articles about hyperthyroidism were selected. Sub-clinical hypothyroidism in early pregnancy, compared with normal thyroid function, was associated with the occurrence of pre-eclampsia [OR 1.7, 95 % CI: 1.1 to 2.6] and an increased risk of perinatal
mortality (OR 2.7, 95% CI: 1.6 to 4.7). In the meta-analyses, the presence of thyroid antibodies was associated with an increased risk of unexplained subfertility (OR 1.5, 95% CI: 1.1 to 2.0), miscarriage (OR 3.73, 95% CI: 1.8 to 7.6), recurrent miscarriage (OR 2.3, 95% CI: 1.5 to 3.5), preterm birth (OR 1.9, 95% CI: 1.1 to 3.5) and maternal post-partum thyroiditis (OR 11.5, 95% CI: 5.6 to 24) when compared with the absence of thyroid antibodies. The authors concluded that pregnant women with subclinical hypothyroidism or thyroid antibodies have an increased risk of complications, especially pre-eclampsia, perinatal mortality and (recurrent) miscarriage.

The Practice Committee of the ASRM (2012) recommended screening for thyroid or prolactin abnormalities for the evaluation and treatment of RPL.

An UpToDate review on “Evaluation of couples with recurrent pregnancy loss” (Tulandi and Al-Fozan, 2013) states that “Thyroid function should be assessed in women with clinical manifestations or a personal history of thyroid disease. Screening asymptomatic women for subclinical thyroid dysfunction is controversial. We feel screening is reasonable since there is evidence of an increased risk of miscarriage in women with subclinical hypothyroidism and in euthyroid women with thyroid peroxidase (TPO) antibodies. Meta-analyses of case-control and cohort studies have found that the presence of TPO autoantibodies in euthyroid women is associated with an increased risk of spontaneous miscarriage that is two to three times higher than in women without these antibodies. In addition, meta-analysis of two randomized trials of the effect of thyroid replacement in these women found treatment was associated with a significant reduction in risk of miscarriage (relative risk 0.48, 95% CI: 0.25 to 0.92); however, there were methodological limitations to these trials”. Moreover, an UpToDate review on “Evaluation of couples with recurrent pregnancy loss” (Tulandi and Al-Fozan, 2013) does not mention measurement of natural killer cells in the evaluation of RPL.

The Practice Committee of the American Society of Reproductive Medicine (ASRM)’s review on “Intravenous immunoglobulin (IVIG) and recurrent spontaneous pregnancy loss” (ASRM, 2006) stated that “For the management of recurrent spontaneous pregnancy loss, IVIG is an experimental treatment”. Furthermore, the Royal College of Obstetricians’ guideline on “The investigation and treatment of couples with recurrent first-trimester and second-trimester miscarriage” (Regan et
al, 2011) stated that "Neither corticosteroids nor intravenous immunoglobulin therapy improve the live birth rate of women with recurrent miscarriage associated with antiphospholipid antibodies compared with other treatment modalities; their use may provoke significant maternal and fetal morbidity".

In a Cochrane review, Porter et al (2006) evaluated the effects of immunotherapy, including paternal leukocyte immunization and intravenous immune globulin on the live birth rate in women with previous unexplained recurrent miscarriages. These investigators searched the Cochrane Pregnancy and Childbirth Group Trials Register (December 2005), the Cochrane Central Register of Controlled Trials (The Cochrane Library 2004, Issue 3), MEDLINE (1966 to September 2004) and EMBASE (1980 to September 2004). Randomized trials of immunotherapies used to treat women with 3 or more prior miscarriages and no more than 1 live birth after, in whom all recognized non-immunologic causes of recurrent miscarriage had been ruled out and no simultaneous treatment was given. The review author and the 2 co-authors independently extracted data and assessed study quality for all studies considered for this review. A total of 20 trials of high quality were included. The various forms of immunotherapy did not show significant differences between treatment and control groups in terms of subsequent live births: paternal cell immunization (12 trials, 641 women), Peto odds ratio (Peto OR) 1.23, 95 % CI 0.89 to 1.70; third party donor cell immunization (3 trials, 156 women), Peto OR 1.39, 95 % CI 0.68 to 2.82; trophoblast membrane infusion (1 trial, 37 women), Peto OR 0.40, 95 % CI 0.11 to 1.45; IVIG, Peto OR 0.98, 95 % CI 0.61 to 1.58. The authors concluded that paternal cell immunization, third party donor leukocytes, trophoblast membranes, and IVIG provided no significant beneficial effect over placebo in improving the live birth rate.

Hogge and colleagues (2007) examined if there is an association between skewed X-inactivation and recurrent spontaneous abortion in a large, well-defined sample of women with recurrent loss. X-chromosome inactivation patterns were compared in 5 groups of women. Group 1 (recurrent spontaneous abortion) consisted of 357 women with 2 or more spontaneous losses. In group 2 (infertility), there were 349 subjects from infertility practices recruited at the time of a positive serum beta-human chorionic gonadotropin. Group 3 (spontaneous abortion) women (n = 81) were recruited at the time of an ultrasound diagnosis of an embryonic demise or an anembryonic gestation. Groups 4 (primiparous) and 5
(multiparous) were healthy pregnant subjects previously enrolled in another study to determine the incidence and cause of pregnancy complications, such as preeclampsia and intrauterine growth restriction. The primiparous group included 114 women in their first pregnancy, whereas the multiparous group consisted of 79 women with 2 or more pregnancies but without pregnancy loss. The rate of extreme skewing (90 % or greater) in the recurrent spontaneous abortion population was 8.6 %, and not statistically different from any of the other groups, except the primiparous group (1.0 %, p < 0.01). The incidence of X-inactivation skewing of 90 % or greater was no different whether there had been at least 1 live birth (9.9 %), or no previous live births and at least 3 losses (5.6 %, p > 0.05). When age and skewing of 90 % or greater were compared, subjects with extreme skewing have a mean age of 2 years older than those without extreme skewing (p < 0.05). The authors concluded that skewed X-inactivation is not associated with recurrent spontaneous abortion but is associated with increasing maternal age.

Warburton and associates (2009) noted that several studies suggested that highly skewed X chromosome inactivation (HSXI) is associated with recurrent spontaneous abortion. These researchers hypothesized that this association reflects an increased rate of trisomic conceptions due to anomalies on the X chromosome that lead both to HSXI and to a diminished oocyte pool. They compared the distribution of X chromosome inactivation (XCI) skewing percentages (range of 50 % to 100 %) among women with spontaneous abortions in 4 karyotype groups: (i) trisomy (n = 154), (ii) chromosomally normal male (n = 43), (iii) chromosomally normal female (n = 38), and (iv) non-trisomic chromosomally abnormal (n = 61) to the distribution for age-matched controls with chromosomally normal births (n = 388). In secondary analyses, these investigators subdivided the non-trisomic chromosomally abnormal group, divided trisomies by chromosome, and classified women by reproductive history. The authors concluded that these data supported neither an association of HSXI with all trisomies nor an association of HSXI with chromosomally normal male spontaneous abortions. Moreover, they also find no association between HSXI and recurrent abortion (n = 45).

Warren et al (2009) estimated genomic copy number changes in fetal loss between 10 and 20 weeks of gestation using array comparative genomic hybridization (aCGH). This was a prospective series of 35 women who
experienced pregnancy loss between 10 to 20 weeks of gestation with either normal karyotype (n = 9) or no conventional cytogenetic testing (n = 26); DNA was isolated from fetal tissue and parental blood. Array comparative genomic hybridization was performed on DNA from fetal tissue using a whole genome BAC array chip. Copy number changes in fetal tissue were then compared against databases of benign copy number changes. Parental DNA was analyzed using the same BAC array in cases that contained suspected pathogenic copy number changes. In cases where de-novo copy number changes were detected in fetal DNA, further characterization was performed using a 244K oligonucleotide microarray. DNA was successfully isolated in 30 of 35 (86 %) of cases. Array comparative genomic hybridization was performed in all of these. De-novo copy number changes were detected in 6 (20 %) cases using the Spectral chip and confirmed in 4 (13 %) cases using the Agilent chip. These ranged in size from 93 to 289 Kb and mapped on 5p, 13q and Xq22. In the cases with de-novo copy number changes, the higher-density Agilent array detected additional changes (20-1,310 Kb). The authors concluded that aCGH detected de-novo copy number changes in 13 % of cases where routine cytogenetic testing was normal or not performed. They stated that these involved large regions of DNA and may provide novel explanations for some cases of otherwise unexplained pregnancy loss.

Kudesia et al (2014) stated that determination of fetal aneuploidy is central to evaluation of RPL. However, obtaining this information at the time of a miscarriage is not always possible or may not have been ordered. These researchers reported on "rescue karyotyping", wherein DNA extracted from archived paraffin-embedded pregnancy loss tissue from a prior dilation and curettage (D&C) is evaluated by aCGH. A retrospective case-series study was conducted at an academic medical center. Patients included had unexplained RPL and a prior pregnancy loss for which karyotype information would be clinically informative but was unavailable. After extracting DNA from slides of archived tissue, aCGH with a reduced stringency approach was performed, allowing for analysis of partially degraded DNA. Statistics were computed using STATA v12.1 (College Station, TX). Rescue karyotyping was attempted on 20 specimens from 17 women. DNA was successfully extracted in 16 samples (80.0 %), enabling analysis at either high or low resolution. The longest interval from tissue collection to DNA extraction was 4.2 years. There was no significant difference in specimen sufficiency for analysis in
the collection-to-extraction interval (p = 0.14) or gestational age at pregnancy loss (p = 0.32). Eight specimens showed copy number variants: 3 trisomies, 2 partial chromosomal deletions, 1 mosaic abnormality and 2 unclassified variants. The authors concluded that rescue karyotyping using aCGH on DNA extracted from paraffin-embedded tissue provided the opportunity to obtain critical fetal cytogenetic information from a prior loss, even if it occurred years earlier. They stated that given the ubiquitous archiving of paraffin embedded tissue obtained during a D&C and the ease of obtaining results despite long loss-to-testing intervals or early gestational age at time of fetal demise, this may provide a useful technique in the evaluation of couples with RPL.

In a meta-analysis, Yang and colleagues (2012) examined the real association in angiotensin converting enzyme insertion/deletion polymorphisms (ACE I/D) gene polymorphisms and RPL. These researchers employed combined PubMed Embase and HuGENet database in data analysis for this meta-analysis from October 2000 to November 2011. The metagen system was used to select the models and effects. Odds ratio with 95 % CI was used to assess the strength of this association. A total of 9 studies from 6 countries with 1,264 RPL and 845 controls were included according to selection criterion. Following the metagen system, these investigators used the dominant model with random effects. The summary OR = 1.61 (95 % CI: 1.10 to 2.36, I (2) = 59.0 %), which suggested the ACE D allele might increase the RPL risk in Asia (OR = 1.97, 95 % CI: 1.31 to 2.98, I (2) = 44.4 %), among Asians (OR = 1.69, 95 % CI: 1.06 to 2.36, I (2) = 32.7 %). In additional, after conducting sensitivity analysis, the results had no differences except for Caucasian subgroup reached to the significance (OR = 2.059, 95 % CI: 1.455 to .914), so these researchers couldn't ignore the relationship between the polymorphisms of ACE D/I gene and Caucasians yet. There seemed no publication bias in the eligible studies with Begg's test (p = 0.867). The authors concluded that results in this meta-analysis presented the positive function of the ACE I/D polymorphism in increasing the RPL risk. Moreover, they stated that furture prospective studies are needed to confirm the precise relationship between the ACE I/D and RPL.

Su and colleagues (2013) stated that a fine balance between coagulation and fibrinolysis is critical in early pregnancy. Angiotensin converting enzyme (ACE) and plasminogen activator inhibitor-1 (PAI-1) are involved
in the fibrinolytic process, and several studies have reported the association between their gene polymorphisms and RPL. These researchers examined the association between ACE and PAI-1 polymorphisms and idiopathic RPL, using meta-analyses. A systematic review of the published literature from the MEDLINE and EMBASE databases before April 2012 was conducted. Of 209 potentially relevant studies, 22 case-control studies comprising a total of 2,820 RPL patients and 3,009 controls were included. Among these studies were 11 reports of 11 of ACE I/D and PAI-1 4G/5G polymorphisms in patients with RPL. A significant association was found with the ACE I/D polymorphism [summary odds ratio 1.29 (95 % CI: 1.02 to 1.62)] in studies including more than 2 recurrent abortions. Subgroup analysis did not show significant associations with RPL in Caucasian and non-Caucasian patients. Meta-analyses of PAI-1 4G/5G polymorphism were not found associations with RPL in studies including more than 2 or 3 recurrent abortions, and in studies of Caucasian and non-Caucasian patients. The authors concluded that meta-analyses showed a significant association between the ACE I/D polymorphism and idiopathic RPL; high clinical heterogeneity existed among studies of PAI-1 4G/5G, and the aggregated data failed to confer higher susceptibility to idiopathic RPL. They stated that more well-designed studies with different ethnic populations are needed for future integration.

Also, UpToDate reviews on “Evaluation of couples with recurrent pregnancy loss” (Tulandi and Al-Fozan, 2014a), “Management of couples with recurrent pregnancy loss” (Tulandi and Al-Fozan, 2014b) and “Definition and etiology of recurrent pregnancy loss” (Tulandi and Al-Fozan, 2014c) do not mention the use of ACE and PAI-1 gene polymorphisms testing.

Wu and colleagues (2012) stated that C677T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) has been associated with recurrent pregnancy loss (RPL). However, results were conflicting. The aim of this study was to quantitatively summarize the evidence for MTHFR C677T polymorphism and RPL risk. Electronic search of PubMed and the Chinese Biomedicine database was conducted to select studies. Case-control studies containing available genotype frequencies of C677T were chosen, and odds ratio (OR) with 95 % confidence interval (CI) was used to assess the strength of this association. The case-
control studies including 2,427 cases and 3,118 controls were identified. The meta-analysis stratified by ethnicity showed that individuals with the homozygous TT genotype had increased risk of RPL (OR = 1.574, 95% CI: 1.163 to 2.13, p = 0.003), in Asians (OR = 1.663, 95% CI: 1.012 to 2.731, p = 0.045). Results among Caucasians did not suggest an association (OR = 1.269, 95% CI: 0.914 to 1.761, p = 0.155). A symmetric funnel plot, the Egger's test (p = 0.285), and the Begg's test (p = 0.529) were all suggestive of the lack of publication bias. The studies conducted in each of the defined number of pregnancy losses -- 2 or more pregnancy losses, and 3 or more pregnancy losses -- showed no effect of the C677T polymorphism on RPL except for the TT versus CT+CT genotype comparison for the 3 or more pregnancy loss subgroup (OR = 1.792, 95% CI: 1.187 to 2.704, p = 0.005). The authors concluded that this meta-analysis supported the idea that MTHFR C677T genotype is associated with increased risk of RPL, except for Caucasians. They stated that to draw comprehensive and true conclusions, further prospective studies with larger numbers of participants worldwide are needed to examine associations between MTHFR C677T polymorphism and RPL.

Hickey et al (2013) stated that MTHFR polymorphism testing is frequently ordered by physicians as part of the clinical evaluation for thrombophilia. It was previously hypothesized that reduced enzyme activity of MTHFR led to mild hyper-homocysteinemia which led to an increased risk for venous thromboembolism, coronary heart disease, and RPL. Recent meta-analyses have disproven an association between hyper-homocysteinemia and risk for coronary heart disease and between MTHFR polymorphism status and risk for venous thrombo-embolism. The authors noted that there is growing evidence that MTHFR polymorphism testing has minimal clinical utility and, therefore should not be ordered as a part of a routine evaluation for thrombophilia.

ACOG guidelines on inherited thrombophilia in pregnancy (2014) state that, because of the lack of association between heterozygosity or homozygosity for MTHFR C677T polymorphism and any negative pregnancy outcomes, including any increased risk for venous thromboembolism, screening with either MTHFR mutation analyses or fasting homocysteine levels is not recommended.
Liu and colleagues (2021) stated that previous studies have suggested that pregnant women with hereditary thrombophilia have a higher risk of RPL, however, the results are inconsistent; furthermore, a complete overview is missing. This lack of information is an obstacle to the risk assessment of RPL in pregnant women with hereditary thrombophilia. In a systematic review and meta-analysis, these researchers examined if there is an association between hereditary thrombophilia in pregnant women and risk of RPL. This review/analysis was carried out using observational studies published in English before April 1, 2020 to assess the relation between hereditary thrombophilia and risk of RPL. Relevant studies were identified from PubMed, Web of Science, and Embase searches and complemented with perusal of bibliographies of retrieved articles. The exposure of interest was hereditary thrombophilia, including factor V Leiden (FVL) mutation, prothrombin gene (PGM), deficiency of antithrombin (AT), deficiency of protein C (PC), and deficiency of protein S (PS). The overall risk estimates were pooled using random effects models. Subgroup and sensitivity analyses were conducted to examine possible sources of heterogeneity and evaluate the robustness of the results. A total of 89 studies involving 30,254 individuals were included. Results showed that women with FVL mutation (OR: 2.44, 95 % CI: 1.96 to 3.03), PGM (OR: 2.08, 95 % CI: 1.61 to 2.68), or deficiency of PS (OR: 3.45, 95 % CI: 1.15 to 10.35) had higher risks of developing RPL. Compared with the reference group, there was no observed relation between a deficiency in AT or PC and RPL (all p > 0.05). Heterogeneity in the risk estimates of RPL was partially explained by geographic region, definitions of RPL, types of RPL, and controlled confounders. Sensitivity analyses validated the robustness of the findings. The authors concluded that the findings of this systematic review and meta-analysis demonstrated a possible association between hereditary thrombophilia and an increased risk of RPL, suggesting that testing for hereditary thrombophilia should be considered in individuals with RPL. Moreover, these researchers stated that he main drawback of this systematic review/meta-analysis was that only 39 of the included studies controlled for 1 or more confounders, and the heterogeneity across all included studies was high. Based on the data available, they could not ascertain if this association was confounded by other potential risk factors of RPL.
Current guidelines from the American Society for Reproductive Medicine (ASRM, 2012) state that the majority of miscarriages are sporadic and are thought to result from genetic causes that are greatly influenced by maternal age. ASRM guidelines recommend peripheral karyotyping of the parents in the evaluation of recurrent pregnancy loss. The guidelines state that peripheral karyotyping is necessary to detect any balanced structural chromosomal abnormalities. Balanced reciprocal translocations and Robertsonian translocations are observed in about 2 % to 5 % of couples with recurrent miscarriage.

Guidelines from the American College of Obstetricians and Gynecologists (2013) concluded that "limited data are available on the clinical utility of chromosomal microarray analysis to evaluate first-trimester and second-trimester pregnancy losses; therefore, this is not recommended at this time."

A literature review by Rajcan-Separovic et al (2010) concluded that the reported use of chromosomal microarray analysis in recurrent pregnancy loss is still limited in comparison with its widespread use to test patients with developmental delay/congenital abnormalities, preimplantation embryos, and continuing pregnancies.

Dhillon et al (2014) reported on a systematic evidence review of chromosomal microarray analysis for diagnosing chromosomal abnormalities in recurrent pregnancy loss. The authors sought to determine whether chromosomal microarray analysis testing on the products of conception following miscarriage provides better diagnostic information compared with conventional karyotyping. The authors identified nine papers meeting inclusion criteria for the systematic evidence review. The authors reported that the papers were heterogenous with both prospective and retrospective methodology. The authors stated that there was wide variation between the papers, including different chromosomal microarray platforms, clinician bias influencing case selection, and indications for referral. The analysis included a relatively small sample size, with 313 cases for overall analysis. Results that were interpreted as variations of unknown significance (VOUS) were grouped with results that were pathogenic. The authors found agreement between chromosomal microarray and karyotyping in 86.0 % of cases (95 % CI:77.0 to 96.0 %). Chromosomal
microarray analysis detected 13 % (95 % CI: 8.0 to 21.0) additional chromosome abnormalities over conventional full karyotyping. However, a portion of these were VOUS. In addition, traditional, full karyotyping detected 3 % (95 % CI: 1.0 to 10 %) additional abnormalities over CMA. The incidence of a VOUS being detected was 2 % (95 % CI: 1.0 to 10.0 %). However, the CIs surrounding this were large, and the authors stated that more data are needed to establish the true VOUS rate. The authors stated that, for purposes of this analysis, cases of VOUS were taken as pathogenic, unless it could be proven to be benign. Therefore, the significance of the microarray detecting clinically and pathogenically significant abnormalities over karyotyping has to be interpreted with caution. The authors stated that the heterogeneity of papers in terms of methodology and study design accounted for the large confidence intervals in their analysis. The authors concluded that further prospective research is needed in this area using large cohort, with a representative, prospective population undergoing both a recognized reproducible array and karyotyping. In addition to this, determining whether the copy number variants found in the products of conception are pathogenic or benign, based on a large cohort of miscarriage cases, is also required.

Guidelines on recurrent pregnancy loss from the American Society for Reproductive Medicine (ASRM, 2012) state that “Currently, routine preimplantation embryo aneuploidy screening is not justified.

For karyotyping of products of conception, van Niekerk et al (2013) stated that “The role of pre-implantational genetic diagnosis (PGD) with in-vitro fertilization (IVF) has been reviewed, and it has been found not to be cost-effective in the management of RPL. In patients with RPL the spontaneous birth rate is still 50 %; with PGD the miscarriage rate may be decreased, but only 33 % of women become pregnant after each PGD/IVF cycle”.

Sahin et al (2014) noted that to select cytogenetically normal embryos, PGD aneuploidy screening (PGD-AS) is used in numerous centers around the world. Chromosomal abnormalities lead to developmental problems, implantation failure, and early abortion of embryos. The usefulness of PGD in identifying single-gene diseases, human leukocyte antigen typing, X-linked diseases, and specific genetic diseases is well-known. In this review, preimplantation embryo genetics, PGD research
studies, and the European Society of Human Reproduction and Embryology PGD Consortium studies and reports were examined. In addition, criteria for embryo selection, technical aspects of PGD-AS, and potential non-invasive embryo selection methods were described. Indications for PGD and possible causes of discordant PGD results between the centers were discussed. The limitations of fluorescence in-situ hybridization, and the advantages of the aCGH were included in this review. Although PGD-AS for patients of advanced maternal age has been shown to improve IVF outcomes in some studies, to the authors’ knowledge, there is insufficient evidence to use advanced maternal age as the sole indication for PGD-AS. The authors concluded that PGD-AS might be harmful and may not increase the success rates of IVF. Moreover, they stated that PGD is not recommended for recurrent implantation failure and unexplained RPL.

Lee et al (2015) examined if PGD for aneuploidy (PGD-A) with analysis of all chromosomes during assisted reproductive technology (ART) is clinically effective and cost-effective? The majority of published studies comparing a strategy of PGD-A with morphologically assessed embryos have reported a higher implantation rate per embryo using PGD-A, but insufficient data has been presented to evaluate the clinical and cost-effectiveness of PGD-A in the clinical setting. These investigators performed a systematic review of the literature for full text English language articles using MEDLINE, EMBASE, SCOPUS, Cochrane Library databases, NHS Economic Evaluation Database and EconLit. The Downs and Black scoring check-list was used to assess the quality of studies. Clinical effectiveness was measured in terms of pregnancy, live-birth and miscarriage rates. A total of 19 articles meeting the inclusion criteria, comprising 3 RCTs in young and good prognosis patients and 16 observation studies were identified; 5 of the observational studies included a control group of patients where embryos were selected based on morphological criteria (matched cohort studies). Of the 5 studies that included a control group and reported implantation rates, 4 studies (including 2 RCTs) demonstrated improved implantation rates in the PGD-A group. Of the 8 studies that included a control group, 6 studies (including 2 RCTs) reported significantly higher pregnancy rates in the PGD-A group, and in the remaining 2 studies, equivalent pregnancies rates were reported despite fewer embryos being transferred in the PGD-A group. The 3 RCTs demonstrated benefit in young and good prognosis
patients in terms of clinical pregnancy rates and the use of single embryo transfer. However, studies relating to patients of advanced maternal age, recurrent miscarriage and implantation failure were restricted to matched cohort studies, limiting the ability to draw meaningful conclusions. The authors concluded that given the uncertain role of PGD-A techniques, high-quality experimental studies using intention-to-treat analysis and cumulative live-birth rates including the comparative outcomes from remaining cryopreserved embryos are needed to evaluate the overall role of PGD-A in the clinical setting. It is only in this way that the true contribution of PGD-A to ART can be understood.

An UpToDate review on “Evaluation of couples with recurrent pregnancy loss” (Tulandi and Al-Fozan, 2015a) does not mention the use of preimplantation genetic diagnosis/PGD as an evaluation tool.

An UpToDate review on “Management of couples with recurrent pregnancy loss” (Tulandi and Al-Fozan, 2015b) states that “In vitro fertilization and preimplantation genetic diagnosis -- Studies evaluating the value of in vitro fertilization (IVF) in women with RPL have yielded mixed results. Embryos of women with unexplained RPL have a higher incidence of aneuploidy for chromosomes 13, 16, 18, 21, 22, X, and Y than embryos obtained from healthy women. However, the value of preimplantation genetic screening in this setting has not been proven ….

Parental Karyotype Abnormality -- Couples with karyotypic abnormalities may choose to undergo prenatal genetic studies, such as amniocentesis or chorionic villus sampling, to determine the fetal karyotype. Pregnancy termination is an option if the fetus is affected. In vitro fertilization (IVF) with preimplantation genetic diagnosis (PGD) can be used to avoid transfer and implantation of an affected embryo. PGD improves the pregnancy outcome of translocation carriers with a history of repeated pregnancy loss. On the other hand, this procedure reduces the live birth rate after IVF if preimplantation testing is performed solely because of advanced maternal age”. Furthermore, PGD and IVF are not mentioned in the “Summary and Recommendations” of this review.

Hyde and Schust (2015) stated that human reproduction is remarkably inefficient; nearly 70% of human conceptions do not survive to live birth. Spontaneous fetal aneuploidy is the most common cause for spontaneous loss, particularly in the first trimester of pregnancy.
Although losses owing to de-novo fetal aneuploidy occur at similar frequencies among women with sporadic and recurrent losses, some couples with RPL have additional associated genetic factors and some have non-genetic etiologies. Genetic testing of the products of conception from couples experiencing 2 or more losses may aid in defining the underlying etiology and in counseling patients about prognosis in a subsequent pregnancy. Parental karyotyping of couples who have experienced RPL will detect some couples with an increased likelihood of recurrent fetal aneuploidy; this may direct interventions. However, the authors noted that the utility of preimplantation genetic analysis in couples with RPL is unproven, but new approaches to this testing show great promise.

Annexin A5 Promoter Haplotype M2 Testing

Nagirnaja et al (2015) noted that annexin A5 is an essential component of placental integrity that may potentially mediate susceptibility to phenotypes of compromised pregnancy. A promoter haplotype termed M2 of the coding gene ANXA5 has been implicated in various pregnancy complications such as preeclampsia and RPL, however with inconclusive results. These researchers carried out a retrospective case-control study combining re-sequencing and restriction fragment length polymorphism (RFLP) analysis in 313 women with unexplained RPL and 214 fertile women from Estonia and Denmark to estimate the RPL disease risk of the M2 haplotype in Northern Europe. Comparative prevalence of the studied ANXA5 genetic variants in human populations was estimated based on the 1000 Genomes Project (n = 675, whole-genome sequencing data) and the KORA S3 500K dataset of South German samples (n = 1,644, genome-wide genotyping data). Minor allele frequency of common polymorphisms in ANXA5 promoter was up to 2-fold lower among Estonian RPL subjects than fertile controls. The M2 haplotype was not associated with RPL and a trend for decreased prevalence was observed among RPL patients compared to controls both in Estonia (8.1 % versus 15.2 %, respectively) and Denmark (9.7 % versus 12.6 %). The high M2 prevalence in fertile controls was consistent with estimations for European and East Asian populations (9.6 % to 16.0 %). The authors concluded that the findings of this study cautioned to consider the M2 haplotype as a deterministic factor in early pregnancy success because: (i) no RPL disease risk was associated with the
haplotype in 2 clinically well-characterized RPL case-control study samples, (ii) high prevalence of the haplotype among fertile controls and world-wide populations is inconsistent with the previously proposed severe impact on early pregnancy success, and (iii) weak impact of M2 haplotype on the production of ANXA5 protein has been established by others.

Estrogen Receptor Beta Gene Polymorphisms Testing

Kim et al (2015) stated that estrogen might play a key role in the maintenance of pregnancy. These investigators examined the role of the estrogen receptor beta (ER-β) gene +1730 G/A, +1082 G/A, and CA repeat polymorphisms in Korean patients with RPL. Genotyping was performed using the TaqMan assay in 305 patients with at least 2 unexplained consecutive spontaneous miscarriages before 20 weeks of gestation and 299 controls. The genotype distributions of the ER-β gene +1082 G/A and +1730 G/A polymorphisms in the RPL group did not differ from those in the control group. When the analysis was restricted to patients with 3 or more consecutive spontaneous miscarriages, there were also no differences in the genotype distribution between this subgroup and controls. The number of CA repeats was distributed from 13 to 28 with 2 large peaks at 18 and 23 in patients with RPL and controls. Using the 2 major peaks as cut-offs, the allele distributions were compared between patients and controls. However, the distribution of ER-β gene CA repeats did not differ between women with recurrent miscarriage and controls. The authors concluded that the findings of this study suggested that the ER-β gene polymorphisms are not major determinants of the development of RPL in Korean women.

Peroxisome Proliferator Activation Receptors (PPARs)

Papamitsou et al (2016) noted that PPAR expression in placenta tissues regulates pro-inflammatory cytokine production and preserves the quiescence of the uterus during pregnancy. PPAR-γ regulates inflammatory response during gestation while PPAR-δ and tumor necrosis factor alpha (TNFα) play a central role at implantation, decidualization and placentation. However, their expression levels affect normal pregnancy and may cause gestational complications and miscarriage. These researchers examined the relationship of these molecules with
unexplained recurrent miscarriage. The miscarriage group was obtained from 12 women, between the ages of 35 to 42 years, who miscarried during the 1st trimester of gestation and controls consisted of 12 healthy women, between the ages of 27 to 39 years, who had electively terminated their pregnancies, during the 1st trimester of gestation. The abortion material was processed and specimens taken were studied using immunohistochemical methods. Specimens were taken from decidua basalis and decidua parietalis. Monoclonal antibodies were used against PPAR-γ (Peroxisome Proliferator Activation Receptor γ), PPAR-δ and TNFα. The results were statistically analyzed with Mann-Whitney test. This study identified PPAR-γ expression in decidua basalis and decidua parietalis from control group and decidua basalis from miscarriage group. PPAR-δ expression was also identified in both deciduas from both groups. Statistically, no significant change in PPAR-γ and PPAR-δ expression was observed between recurrent miscarriage group and controls. On the contrary, a statistically significant up-regulation of TNFα was identified in both deciduas between miscarriage group and controls (p < 0.05). The authors concluded that the evidence did not support a possible role of PPARs expression in RPL. However, a potential involvement of TNFα in the syndrome was reported. They stated that further research should be performed due to insufficient bibliographic data.

Miscellaneous Experimental Diagnostic Procedures

An UpToDate review on “Evaluation of couples with recurrent pregnancy loss” (Tulandi and Al-Fozan, 2016) states the following:

- Autoantibodies and immune function -- Many studies have reported the presence of autoantibodies in women with RPL. Only anticardiolipin antibody and lupus anticoagulant have been clearly associated with pregnancy loss. The pregnancy outcome of women with and without antinuclear antibody (ANA) is the same; available data do not support testing women with RPL for ANA. With the exception of anticardiolipin antibody and lupus anticoagulant, we recommend not testing women with known autoimmune diseases or unexplained RPL for autoantibodies for the purpose of attempting to predict their risk of pregnancy loss.
- Selection of appropriate tests for diagnosis of immune-based RPL also requires further investigation and validation. Results from HLA typing, mixed lymphocytotoxic antibody tests and mixed lymphocyte culture reactions are not predictive of pregnancy outcome. The role of differences in the CD56+ population of cells and alterations in cytokines produced by monocytes, CD4+ cells, and endometrium remains investigational.

- Progesterone level — Single or multiple serum progesterone levels are not predictive of future pregnancy outcome.

- Endometrial biopsy -- Diagnosis of a luteal phase defect had been based upon results of endometrial biopsy. However, high quality data show that this test is not predictive of fertility status; therefore, it is no longer recommended.

Rasmek Roepke and co-workers (2018) stated that medical treatment of women with idiopathic RPL is controversial. These researchers examined the effects of different treatments on live-birth rates (LBRs) and complications in women with unexplained RPL. They searched Medline, Embase and the Cochrane Library, and identified 1,415 publications. This systematic review included 21 RCTs regarding acetylsalicylic acid, LMWH, progesterone, IVIG or leukocyte immune therapy in women with 3 or more consecutive miscarriages of unknown cause. The study quality was assessed and data were extracted independently by at least 2 authors. No significant difference in LBR was found when acetylsalicylic acid was compared with LMWH or with placebo. Meta-analyses of LMWH versus control found no significant differences in LBR [risk ratio (RR) 1.47, 95 % CI: 0.83 to 2.61]. Treatment with progesterone starting in the luteal phase appeared effective in increasing LBR (RR 1.18, 95 % CI: 1.09 to 1.27) but not when started after conception; IVIG showed no effect on LBR compared with placebo (RR 1.07, 95 % CI: 0.91 to 1.26). Paternal immunization compared with autologous immunization showed a significant difference in outcome (RR 1.8, 95 % CI: 1.34 to 2.41), although the studies were small and at high-risk of bias. The authors concluded that available literature does not allow advice on any specific treatment for idiopathic RPL, with the exception of progesterone starting from ovulation. These investigators suggested that any treatment for RPL should be used within the context of a RCT.

Progesterone Treatment
Coomarasamy et al (2015) stated that progesterone is essential for the maintenance of pregnancy. However, whether progesterone supplementation in the first trimester of pregnancy would increase the rate of live births among women with a history of unexplained recurrent miscarriages is uncertain. These researchers conducted a multi-center, double-blind, placebo-controlled, randomized trial to examine if treatment with progesterone would increase the rates of live births and newborn survival among women with unexplained recurrent miscarriage. They randomly assigned women with recurrent miscarriages to receive twice-daily vaginal suppositories containing either 400 mg of micronized progesterone or matched placebo from a time soon after a positive urinary pregnancy test (and no later than 6 weeks of gestation) through 12 weeks of gestation. The primary outcome was live birth after 24 weeks of gestation. A total of 1,568 women were assessed for eligibility, and 836 of these women who conceived naturally within 1 year and remained willing to participate in the trial were randomly assigned to receive either progesterone (404 women) or placebo (432 women). The follow-up rate for the primary outcome was 98.8 % (826 of 836 women). In an intention-to-treat analysis, the rate of live births was 65.8(488) % (262 of 398 women) in the progesterone group and 63.3 % (271 of 428 women) in the placebo group (relative rate, 1.04; 95 % CI: 0.94 to 1.15; rate difference, 2.5 percentage points; 95 % CI: -4.0 to 9.0). There were no significant between-group differences in the rate of adverse events. The authors concluded that progesterone therapy in the first trimester of pregnancy did not result in a significantly higher rate of live births among women with a history of unexplained recurrent miscarriages.

Interleukin Genes Polymorphisms

Chen et al (2015) stated that interleukin-18 (IL-18) plays a potential pathological role in RPL. The results of published studies on the relationship between IL-18 gene promoter polymorphisms (-137G/C and-607C/A) and RPL risk remain controversial. These investigators performed a meta-analysis was to evaluate the association of IL-18, -137G/C and-607C/A gene polymorphisms with the risk of RPL under recessive, dominant and additive genetic models. A literature search was conducted in Medline, Embase and Web of Science for studies that described the effect of IL-18 gene polymorphisms on RPL risk. The numbers of each -137G/C and-607C/A genotype in the case and control
groups were extracted. Quality of the original studies' methodology was also assessed. Meta-analysis was performed using Stata 13.1 software and the fixed effect model was used. A total of 5 articles were included in this meta-analysis. No significant heterogeneity between the studies was noted. The IL-18 -137 G/C polymorphism was significantly associated with an increased risk of RPL under a recessive genetic model (CC versus GG + CG: OR = 1.56, 95 % CI: 1.13 to 2.15). For the -607C/A mutation, these researchers failed to find any association under any genetic models. The Egger's regression asymmetry test showed no publication bias. The authors concluded that the findings of the study indicated a positive association between the CC genotype of the IL-18 -137G/C gene and RPL risk. They stated that future well-designed large studies are needed to validate the association between IL-18 gene polymorphisms and the risk of RPL.

Zhang and colleagues (2017) noted that ILs are a group of immunomodulatory proteins that mediate a variety of immune reactions in the human body. These investigators examined the association between IL gene polymorphisms and RPL. They reviewed 21 studies from Medline, Embase, OVID SP and PubMed to evaluate RPL-related IL gene polymorphisms. Meta-analysis was performed on 12 of the polymorphisms, and a review included the others. The integrated results indicated that IL-1β (-511C/T) (p = 0.02, 95 % CI: 0.77 [0.62 to 0.96]), IL-6 (-634C/G) (p < 0.001, 95 % CI: 2.91 [2.01 to 4.22]), IL-10 (-1082G/A, -819T/C) (p = 0.01, 95 % CI: 0.80 [0.67 to 0.96]; p < 0.01, 95 % CI: 0.66 [0.49 to 0.89]), and IL-18 (-137G/C, -105G/A) (p < 0.01, 95 % CI: 1.69 [1.24 to 2.31]; p = < 0.01, 95 % CI: 1.41 [1.17 to 1.70]) consistently associated with RPL after meta-analysis. IL-17A rs2275913 and IL-17F rs763780, IL-21 rs2055979 and rs13143866, IL-1β (-31C/T), IL-6 (-2954G/C), and IL-10 (-536A/G) were reported only once as having a significant association with RPL. The authors concluded that although these polymorphisms contain only 1 base change, the findings of this study added further evidence indicating that RPL is a polygenic disease, implying that these polymorphisms are potential markers for RPL.
Li and colleagues (2016) noted that several studies on the association of TNF-α polymorphisms with RPL risk have reported conflicting results. These researchers carried out a meta-analysis to provide a more precise estimation of these relationships and examined the real association between TNF-α polymorphisms and RPL. An extensive eligible literature search for relevant studies was conducted on PubMed, Embase, and the Cochrane Library from their inceptions to May 12, 2015. Specific inclusion criteria were used to evaluate articles. The OR with 95% CIs were used to assess the strength of associations. Statistical analyses were performed by the STATA12.0 software. A total of 10 case-control studies including 1,430 RPL patients and 1,727 healthy controls were identified. Meta-analysis indicated that TNF-α-308G/A (rs1800629) polymorphism in the TNF-α gene correlated with elevated RPL risk whereas no significant association was observed between TNF-α-238G/A (rs361625) and RPL. The authors concluded that this meta-analysis evaluated the association between TNF-α genetic polymorphisms and RPL risk and demonstrated that -308G/A polymorphism in the TNF-α gene is associated with susceptibility to RPL. They stated that this polymorphism might be a risk factor for RPL; and larger and well-designed studies (including functional studies) are needed to re-evaluate the association between TNF-α gene and RPL.

This study had several major drawbacks: (i) the number of studies and the sample sizes were relatively small for analysis of each gene polymorphism thereby having insufficient power to estimate the association between TNF-α genetic polymorphisms and RPL risk, (ii) a meta-analysis is a retrospective study, the selection bias would lead to the heterogeneity of the results, and thereby possibly influencing the reliability of the conclusions, (iii) even though the studies had similar inclusion criteria, there were also some differences such as different examinations of each group patients, potential confounders (i.e., age, race) might skew the results, and (iv) this study only included articles published in English from the 3 selected databases, which might limit the results of the meta-analysis. It was critical that larger and well-designed studies should be performed to re-evaluate the association precisely.

Mitochondrial DNA Variations Analysis
Azadi and associates (2018) stated that cases with 3 or more consecutive spontaneous abortions before the 20th week of gestation are termed as RPL. Problems in implantation of the fetus and any retarded growth of the fetus in the uterus can be correlated to RPL. Possible causes of RPL would include the genetic variations in the regulatory enzymes of the crucial metabolic pathways, clotting factors, hormones and hormone receptors. This defect of the mitochondrial respiratory chain is recognized as a major cause of human disease. These researchers examined 73 women with RPL and 100 healthy normal controls. By using the direct sequencing method, the amplified products including the mtDNA complex I genes were analyzed. Overall, 7 variations in mitochondrial complex I genes were found (T4216C, A5153G, C10142T, C12062T, A12662G, G14179A and T14263C) using direct sequencing technique. The RPL group had significantly higher proportions of the different variants than those observed of the control group. The authors concluded that more research is needed to understand the effect and role of the mitochondrial variations in the progress of RPL, which may vary among individuals and different ethnic groups.

Prolactin Receptor Gene Polymorphism Testing

In a case-control study, Kim and colleagues (2018) examined the role of the prolactin receptor gene C/T polymorphism in 311 Korean women with RPL and 314 controls. Genotyping for prolactin receptor gene intron C/T polymorphism was performed using a TaqMan assay. The significance of difference in the genotype distribution was assessed using a Chi-square test, and continuous variables were compared using a Student's t-test. The genotype distribution of the prolactin receptor gene C/T polymorphism in the RPL group did not differ from that in the control group (CC/CT/TT rates were 49.8 %/41.5 %/8.7 % and 52.5 %/37.6 %/9.9 % for the RPL patient and control groups, respectively, p = 0.587). When the analysis was restricted to patients with 3 or more consecutive spontaneous miscarriages or patients without prior live-birth, there were also no differences in the genotype distribution between these subgroups and controls. The authors concluded that the findings of the current study suggested that the prolactin receptor gene intron C/T polymorphism was not a major determinant of the development of RPL. Many studies have examined if there is a genetic component for the risk of RPL. Recently, 1 study examined if genetic polymorphisms involved in the regulation of the
hypothalamic-pituitary-ovarian axis would be associated with recurrent miscarriage. Among 35 polymorphisms in 20 candidate genes, genotype distribution with regard to the prolactin receptor gene intron C/T polymorphism (rs37389) differed between the recurrent miscarriage and the control groups. Since this study reporting the candidate association between the prolactin receptor gene and recurrent miscarriage, no replication study has been performed. The genotype distribution of the prolactin receptor gene C/T polymorphism in the recurrent miscarriage group did not differ from that in the control group. The authors stated that the findings of this study may be useful in that it is the first replication study since the initial report of the association of prolactin receptor gene polymorphism with recurrent miscarriage. They noted that although no association was found, the potential role of prolactin in pregnancy loss needs to be further investigated because prolactin and its receptor have been postulated to play an important role in the maintenance of normal pregnancy.

Serum Anti-Heat Shock Protein Antibodies Levels

Matsuda and associates (2017) noted that vascular dysfunction has been reported in women with RPL. These investigators examined the severity of vascular dysfunction in non-pregnant women with RPL and its correlation with anti-heat shock protein (HSP) antibodies that are known to induce arteriosclerosis. They measured the serum anti-HSP60 antibodies, anti-HSP70 antibodies, and anti-phospholipid antibodies (APA) in 68 women with RPL and 29 healthy controls. Among the women with RPL, 14 had a diagnosis of antiphospholipid syndrome (APS), and in the remaining 54, the causes for RPL were unexplained. Compared to the controls, the brachial-ankle pulse wave velocity (baPWV), carotid augmentation index (cAI), and uterine artery pulsatility index (PI) were all significantly higher in the women with both APS and unexplained RPL. Compared to the controls, the anti-HSP60 antibody levels were significantly higher in the APA-positive group of women with unexplained RPL, and the anti-HSP70 antibody levels were significantly higher in APS and APA-positive group of women with unexplained RPL. However, the anti-HSP60 and anti-HSP70 antibody levels did not correlate with the values of baPWV or cAI. The authors concluded that these findings showed that anti-HSP60 and anti-HSP70 antibodies were increased in
women with unexplained RPL. They stated that further studies are needed to elucidate the roles of anti-HSP antibodies and their pathophysiology in unexplained RPL.

Ovarian Reserve Tests (e.g., Antral Follicle Count and Serum Anti-Mullerian Hormone Levels)

In a retrospective, cohort study, Pils and colleagues (2017) correlated anti-Mullerian hormone (AMH) levels and other parameters for ovarian reserve to the gestational age at the time of pregnancy loss in women with idiopathic RM. A total of 79 patients had suffered a total of 266 miscarriages. When comparing women with an "unembryonic" to those with an "embryonic" most recent miscarriage, there was no difference in median age (36.3 years, inter-quartile range [IQR] of 31.6 to 40.1 versus 34.2 years, IQR of 29.9 to 38.0; p = 0.303); but in median AMH levels (0.7, IQR of 0.2 to 18, versus median 1.8, IQR of 1.3 to 3.3, respectively, p = 0.044) and in the rate of patients with an AMH less than or equal to 1 ng/ml (23/37, 62.2 %, versus 8/42, 19 %; p < 0.001). The authors concluded that AMH might add to the diagnostic process in RM in the future. They stated that to their best knowledge, this was the first study to compare markers of ovarian reserve with age at pregnancy loss in women with RM. These findings suggested that AMH might be a valuable parameter in addition to chronological age in the future. However, these investigators stated that they could not provide reliable data on these issue in the present study because of its retrospective design and the small sample size (n = 79) that must be considered general study limitations. They stated that larger prospective trials are needed to prove the finding that lower AMH levels were associated with earlier gestational age at miscarriage and to further evaluate the value of AMH in the diagnostic process of RM.

In a systematic review and meta-analysis, Bunnewell and colleagues (2020) examined the association between diminished ovarian reserve (DOR) in women at risk of RPL using ovarian reserve tests. These researchers carried out a systematic review of major electronic databases including Medline, Embase, Web of Science, and Scopus for studies that examined the incidence of DOR in women with RPL. Main outcome measure was association between RPL and DOR. In studies up to May 2019, these investigators examined quality using the Newcastle-Ottawa
Scale and meta-analyzed data using a random-effect model. They included 15 studies \( n = 3,082 \) women reporting on 6 ovarian reserve tests: anti-Mullerian hormone [AMH], antral follicle count (AFC), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and FSH:LH ratio. More women with RPL appeared to have DOR compared with women who did not have RPL as measured by low AMH levels (OR 2.77; 95% CI: 1.41 to 5.46) and AFC (OR 2.45; 95% CI: 1.16 to 5.19). Women with unexplained RPL also appeared to have a higher association with DOR compared with women whose RPL had a known etiology, as measured by low AMH levels (OR 3.23; 95% CI: 1.81 to 5.76). No statistically significant differences were observed in the levels of any of the remaining ovarian reserve tests between those groups of women. The authors concluded that there was an apparent association between DOR and RPL; low AMH and AFC levels could predict higher odds for pregnancy loss. Moreover, these researchers stated that more studies are needed to examine their prognostic value in the management of women with RPL. These investigators also noted that future large prospective cohort studies are also needed to examine the role of ovarian reserve tests screening for subfertility in clinical practice and to identify the test with the best cost-effective qualities.

Immunotherapy for Recurrent Pregnancy Loss

In a systematic review and meta-analysis, Achilli and colleagues (2018) examined the evidence on the role of immunotherapy in IVF and in the management of RPL. These investigators carried out a literature search using Medline, PubMed, CINAHL, and Embase until May 2017. Only RCTs were included, and a meta-analysis was performed where appropriate. Subjects were women undergoing IVF treatment with or without a history of recurrent implantation failure and women with idiopathic RPL. These investigators evaluated the efficacy of commonly used immuno-modulators such as IV use of immunoglobulin, lymphocyte immunotherapy and intra-lipid; intra-uterine infusion of granulocyte colony-stimulating factor (G-CSF) and peripheral blood mononuclear cells; subcutaneous administration of TNF-alpha inhibitors, leukemia inhibitory factor; and oral administration of glucocorticoids. The primary outcomes were live-birth rate and miscarriage rate; secondary outcome was clinical pregnancy rate. Of the 7,226 publications identified, 53 were selected during the initial screening; 30 satisfied the selection criteria and
were included in this review. The authors concluded that available
evidence showed controversial results regarding the role of
immunotherapy when used for improving reproductive outcomes. This
study did not show a role for immunotherapy in improving the live-birth
rate in women undergoing IVF treatment or in the prevention of idiopathic
RPL. These researchers stated that currently, immunotherapy should be
used in the context of research and should not be used in routine clinical
practice to improve reproductive outcomes.

IVIG

The Canadian Agency for Drugs and Technologies in Health’s review on
“Off-label use of intravenous immunoglobulin for recurrent spontaneous
abortion” (No authors listed, 2018) concluded that “Evidence informing
the use of IVIG for patients with recurrent spontaneous abortion (RSA) is
inconsistent with regard to its effect on live-birth. While 4 studies in this
review suggested a benefit of IVIG for patients with RSA, 5 studies of
higher quality found no effect. All of the included studies demonstrated
some risk of bias due to uncertain external validity (representativeness)
and some studies demonstrated a risk of confounding, in particular. One
systematic review reported a beneficial effect of IVIG with marginal
statistical significance, but cautioned the interpretation of their findings
due to the impact of one or more included primary studies. Consequently,
the findings summarized within this review should be interpreted with
caution, and the clinical effectiveness of IVIG for RSA remains unclear.
Further evidence from larger, more robust studies -- particularly those that
focus on particular subgroups of RSA patients -- remains necessary to
reduce uncertainty”.

In-Vitro Fertilization-Preimplantation Genetic Diagnosis for
Improvement of Reproductive Outcome in Couples with Recurrent
Pregnancy Loss

Maithripala and colleagues (2018) noted that parental carriers of
balanced structural chromosomal re-arrangements such as reciprocal or
Robertsonian translocations are at increased risk of RPL due to the
production of gametes with unbalanced non-viable chromosome variants.
As a purported means of improving reproductive outcomes in this
population, IVF and PGD have been introduced as an alternative to
natural conception and prenatal diagnosis. In a retrospective, chart review, these investigators examined the prevalence and therapeutic options of couples with structural chromosomal re-arrangement referred to a tertiary care RPL clinic. Furthermore, these researchers compared the 2 methods of management in terms of LBR. This trial entailed 2,321 couples who were referred to a highly specialized RPL clinic for ongoing clinical management between January 2005 and December 2013 (n = 23); couples who pursued PGD through local fertility centers during this time were also included (n = 13). A total of 36 couples (1.6 %) were found to be parental carriers of a structural chromosomal re-arrangement. In this cohort, couples were twice as likely to pursue natural conception compared with IVF with PGD. No significant differences were observed in LBR between PGD and clinical management (66.6 % versus 53.3 %, p = 0.717). With PGD management, 6 live-birth outcomes were observed, with an incidence of 1 birth in 5.63 years of follow-up. With clinical management, 24 live-birth outcomes were observed, with an incidence of 1 birth in 4.09 years of follow-up. Mean time to live-birth was 17.5 months and 23.3 months in clinical management and PGD, respectively. The authors concluded that among couples presenting to a tertiary RPL clinic, parental carriers of structural chromosomal re-arrangement and history of RPL were more likely to pursue natural conception over IVF and PGD. With regards to reproductive outcomes, no significant difference in miscarriage rate, time to live-birth, or LBR was observed between couples who pursued PGD compared with expectant clinical management.

Iews and associates (2018) stated that RPL is a common, yet elusive, complication of pregnancy. Among couples at high-risk of RPL, such as those carrying a structural chromosomal re-arrangement, PGD has been proposed as a tool to improve live-birth rates and reduce the incidence of miscarriage; however, no clear consensus has been reached on its benefits in this population. In a systematic review, these investigators summarized existing published research on the effect of PGD on pregnancy outcomes among carriers of chromosomal abnormalities with RPL. A comprehensive search of common databases was conducted, which yielded 20 studies. Meta-analysis was precluded owing to significant heterogeneity between studies. The primary outcome of interest was LBR, and a pooled total of 847 couples who conceived naturally had a LBR ranging from 25 to 71 % compared with 26.7 to 87 % among 562 couples who underwent IVF and PGD. The authors
concluded that patients experiencing RPL with structural chromosomal rearrangement should be counselled that good reproductive outcomes could be achieved through natural conception, and that IVF-PGD should not be offered 1st-line, given the unproven benefits, additional cost and potential complications associated with ART. These researchers stated that drawbacks of this study included lack of large comparative or RCTs.

Placenta-Specific Protein1 (PLAC1)

Yilmaz and colleagues (2020) noted that observations from studies have provided evidence that placenta-specific protein1 (PLAC1) is important for the establishment and maintenance of pregnancy and suggested it as a potential biomarker for gestational pathologies. These investigators examined if maternal serum PLAC1 levels have any impact on etiopathogenesis of PL and repeated implantation failure after IVF. They conducted a prospective, observational, case-control study in which 28 patients with RPL (group 1), 30 patients with unexplained infertility and IVF (group 2), 29 fertile patients (group 3) were included. The demographic features and serum PLAC1 levels were compared. There was a significant difference in PLAC1 levels between the groups (group 1 = 19.71 ± 16.55 ng/ml; group 2 = 4.82 ± 1.44 ng/ml; group 3 = 0.89 ± 0.62 ng/ml, respectively) (p = 0.001). Positive correlation was found between serum PLAC1 levels and abortion rates (r = 0.64; p = 0.001), a negative correlation was found between serum PLAC1 levels and live-birth rates (r = -0.69; p = 0.001). PLAC1 might have a negative effect on implantation in RPL and IVF. There may be a subgroup of PLAC with different bioactivity. There were no relevant studies conducted among these populations, further large-scale studies are needed to assess the molecular role of PLAC1 on implantation. These researchers noted that PLAC1 (placenta-specific protein-1) gene is located on the X chromosome that encodes for a protein that is thought to be important for placental development although its role has not been clearly defined.

Studies in the literature have provided evidence that PLAC1 has an important role in the establishment and maintenance of pregnancy and suggested it as a potential biomarker for gestational pathologies. Several reports over the past few years have demonstrated PLAC1 expression in a variety of human tumors including lung cancers, breast cancer, hepatocellular and colorectal cancers, gastric cancers and uterine cancers. There have been no previous studies conducted among
patients with RPL or repeated implantation failure after IVF that have searched for any association between PLAC1 levels and implantation failure. This study has demonstrated higher PLAC1 levels in infertile women with IVF and RPL for the first time; suggesting that it could have a negative effect on implantation in these populations. PLAC1 could be detected in the serum as a biomarker that is associated with IVF and RPL. The authors stated that defining the precise role of PLAC1 during implantation will provide new insight into understanding of poor reproductive outcomes such as IVF and RPL and help in developing treatment strategies. These researchers stated that further large-scale studies with more patients are needed to uncover the clinical value of PLAC1 as a biomarker to predict repeated implantation failure and RPL.

Placental Expression of Pentraxin 3 (PTX3)

Zeybek and colleagues (2019) stated that pentraxin 3 (PTX3), a prototypical member of the long pentraxin subfamily, is a evolutionarily conserved multimeric pattern recognition receptor involved in the humoral component of the innate immune system. Pentraxin 3 is released when tissue is stressed or damaged, and interacts with many different ligands. Pentraxin 3 exerts a pivotal role both as a regulator and as an indicator of inflammatory response in the pathogenesis of many diseases such as sepsis, vasculitis and preeclampsia. Uncontrolled inflammatory response is considered a major cause of unexplained RPL (URPL). These researchers determined the PTX3 messenger ribonucleic acid (mRNA) and protein expression levels in placental tissues from 50 women with URPL, and made comparison with those in 50 age-matched control subjects. In quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemistry analyses, PTX3 mRNA and protein levels, respectively, were significantly increased in URPL patients compared with their respective controls (p = 0.0001). Although no significant correlations were identified between PTX3 expression levels and clinical parameters such as maternal age, numbers of previous pregnancy losses, and gestational age at miscarriage, PTX3 mRNA expression was significantly higher in patients with no live-births than in women with previous live-births (p = 0.0001). The authors concluded that the findings of this study suggested that tissue-specific expression of PTX3 was associated with URPL. These researchers stated that further, larger studies (including functional studies that examine the interactions between PTX3 and other
immunological signaling molecules that are stimulated under the conditions of URPL) are needed to examine if PTX3 expression can be used as a biomarker to manage URPL in routine clinical practice.

Sperm DNA Fragmentation

Carlini and co-workers (2017) stated that the etiopathogenesis of RPL is heterogeneous. These investigators examined the male factor in Italian couples experiencing RPL following natural conception. The study examined 112 men from RPL couples and 2 control groups: 114 infertile men with 1 or more impaired semen parameters and 114 fertile men with high-quality semen parameters. Semen parameters were examined according to WHO criteria. Sperm DNA fragmentation (sDF) was evaluated using TdT-mediated dUDP nick-end labelling (TUNEL) assay. With the exception of ejaculate volume, the seminal profile of patients with RPL was similar to that of fertile patients and better than the infertile ones. Despite good spermatogenesis, however, sperm DNA integrity was impaired in the RPL group, with sDF values significantly higher than in fertile controls (18.8 ± 7.0 versus 12.8 ± 5.3, p < 0.001) and similar to those of infertile patients; sDF also showed a positive correlation with the age of patients with RPL and number of miscarriages. The authors concluded that the findings of this study suggested a correlation between increased sDF and impaired reproductive capacity in terms of both fertilization and pregnancies carried to term, however, high sDF cannot yet be considered a predictive factor for the risk of RPL.

In a systematic review and meta-analysis, McQueen and colleagues (2019) examined the rate of sDF in male partners of women with RPL and fertile control women. A total of 579 male partners of women with RPL and 434 male partners fertile control women were included in this review. Prospective studies were identified through a PubMed search; RPL was defined as 2 or more previous pregnancy losses. Fertile control women had a history of a live-birth or ongoing pregnancy. The primary outcome was the rate of sDF. The summary measures were reported as mean difference (MD) with 95% CI. A total of 15 prospective studies were included in a qualitative review. Pooled data from 13 studies with sufficient data for meta-analysis suggested that male partners of women with a history of RPL have a significantly higher rate of sDF compared to the partners of fertile control women: MD 11.91, 95% CI: 4.97 to 18.86.
The authors concluded that these findings supported an association between sDF and RPL. Moreover, these researchers stated that given the significant heterogeneity between studies and lack of prospective pregnancy outcome data, further large prospective studies are needed.

Tan and associates (2019) noted that sDF has emerged as a valuable tool for evaluating male fertility, yet the relationship between DNA fragmentation in the male gamete and idiopathic RPL remains a topic of ongoing debate. These researchers carried out a meta-analysis of 12 prospective and 2 retrospective studies involving 530 men with a history of RPL who underwent sDF testing compared with 639 fertile control participants. The main outcome measures were sDF measured by comet assay, TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (TUNEL), sperm chromatin dispersion (SCD) or sperm chromatin structure assay. Overall, couples with a history of idiopathic RPL demonstrated higher levels of sDF than fertile couples (average MD 11.98, p < 0.001). Subgroup analysis demonstrated a similar average MD between the RPL and control groups using SCD compared with TUNEL, while mean paternal age and mean sperm motility in the RPL groups tested by meta-regression demonstrated no significant effect on the MDs in sDF (p > 0.10). The authors concluded that these findings supported the diagnostic value of sDF over standard semen analysis, as well as a possible paternally derived genetic origin of unexplained RPL. Moreover, these researchers stated that further corroboration and large, prospective, randomized studies are needed to examine the predictive utility of sDF for assessing couples with unexplained RPL.

Furthermore, an UpToDate review on “Evaluation of couples with recurrent pregnancy loss” (Tulandi and Al-Fozan, 2020) states that “Male contribution to RPL is still unclear. Sperm DNA fragmentation has been associated with miscarriage. However, with the exception of the karyotype analysis, no other testing is recommended for the male partner of a woman with RPL”.

Expression of Pinopodes in the Endometrium: Role of Thrombosomodulin and Ezrin
D'Ippolito and colleagues (2020) noted that pinopode expression has been suggested as a marker of endometrial receptivity. These researchers set up an experimental study comparing endometrial tissue from RPL (n = 30) and fertile control (CTR, n = 20) women in terms of pinopode expression/morphology; expression of thrombomodulin (TM) and ezrin; cytoskeletal organization. Endometrial samples were collected during implantation window and evaluated by scanning electron microscopy, Western blot, and immunofluorescence. They found that RPL endometrial tissue showed increased pinopodes density (p < 0.05); a reduced diameter of pinopodes (p < 0.05); a decreased TM and ezrin expression (p < 0.05). Furthermore, confocal images showed a significantly reduced expression of phosphorylated (p)-ezrin, confirming the results obtained through immunoblot analysis. Immunofluorescence staining showed that in CTR samples, junctions between cells were intact and clearly visible, whereas actin filaments appeared completely lost in RPL endometrial samples; suggesting that due to the impaired expression and activity of TM and ezrin, actin did not bind to plasma membrane in order to orchestrate the cytoskeletal actin filaments. The authors concluded that these findings suggested that an impaired expression of TM and expression/activation of ezrin may affect the connection between the TM and actin cytoskeleton, impairing the organization of cytoskeleton and, eventually, the adequate pinopode development.

These researchers demonstrated that a down-regulation of TM-ezrin complex is involved in the alteration of cytoskeletal organization in endometrial cells and that such mechanisms impact pinopode development. They stated that further studies on human endometrium are needed in order to better define the best receptive endometrium. In addition, these investigators previously demonstrated an increased unfavorable expression of inflammatory cytokines and NACHT-LRR-PYD domains-containing protein 3 (NALP-3) inflammasome in the endometrium from RPL women. Furthermore, experiments in mouse models of renal interstitial inflammation reported the ability of TM to neutralize the inflammatory response by inhibiting NALP-3 activity. These findings led them to hypothesize possible connections between these 2 complexes. They stated that further studies on human endometrium are needed in order to examine a possible communication between these 2 systems at the endometrial level, even in RPL women. The
demonstration that a correlation between the 2 systems exists also at an endometrial level might aid in outlining an endometrial set-up unfavorable to the implantation process and thus in selecting conditions of altered endometrial status.

Igenomix POC (Products of Conception) Test

The POC (Products of Conception) test by Igenomix is an advanced analysis of fetal tissue using next generation sequencing (NGS) technology that screens the 46 chromosomes and examines if a chromosomal abnormality is present.

Dai and associates (2019) noted that fetal chromosomal abnormalities are considered to be the main cause of spontaneous abortion (SA). These researchers examined the differences in the rates and numbers of chromosomal abnormalities between samples from women with a history of 1 versus more than 1 SA as well as between samples from 1st- and 2nd-trimester SAs in women from Northeast China. A total of 1,210 POCs from patients with a history of 1 or more SAs were examined. Of these 1,210 samples, 434 were from women with a history of 1 SA, and 776 were from women with a history of more than 1 SA. Furthermore, 1,071 samples were from the 1st trimester, 118 were from the 2nd trimester, and 21 were from the 3rd trimester. These investigators identified chromosomal abnormalities by NGS technology. Among the 1,210 POCs in women with SA, 607 (50.17 %) had fetal chromosomal abnormalities. There were no significant differences in the rates of chromosomal abnormalities according to the abortion frequency. However, 1st-trimester SA had a significantly higher percentage of fetal chromosomal abnormalities than 2nd-trimester SA (p < 0.05). Among 663 chromosomal abnormalities, 633 abnormalities occurred in 1st-trimester SA; the most frequent karyotype was trisomy 16 (14.38 %), followed by monosomy X (13.27 %), trisomy 22 (7.90 %), and trisomy 15 (5.37 %); 30 abnormalities occurred in 2nd-trimester SA; the most frequent karyotype was trisomy 18 (26.67 %), followed by monosomy X (16.67 %), trisomy 21 (13.33 %), and trisomy 13 (10.00 %). No chromosomal abnormalities occurred in the 3rd trimester. These researchers stated that these findings showed the importance of determining the genetic cause of abortion in patients with a history of SA.
They also identified a trend suggesting that the percentage of fetal chromosomal abnormalities was significantly higher in 1st- than 2nd-trimester SA. The authors concluded that the detection rate of chromosomal abnormalities in POCs from SA could be increased by NGS, which is beneficial for couples with recurrent miscarriages and offers better genetic counseling in the clinical setting. Moreover, these researchers noted that patients in the 1st trimester had a significantly higher percentage of chromosomal abnormalities than those in the 2nd trimester, and no chromosomal abnormalities of POCs were found in the 3rd trimester. They stated that further research is needed because of the limited number of samples in this study. These investigators speculated that the cause of abortion in the 3rd trimester had little to do with fetal chromosomal abnormalities, and other tests are needed to identify the cause.

Xu and colleagues (2020) stated that miscarriage is the spontaneous loss of a clinically established intra-uterine pregnancy before the fetus has reached viability. In order to compare the performance of traditional G banding karyotyping with NGS for detecting common trisomies in POC. Chromosome abnormalities were detected by high-resolution G banding karyotyping (G-banding) and NGS. A total of 48 miscarriage samples, including 20 samples without karyotype result and 28 with karyotype results were selected and coded for analysis by NGS. The multiplex PCR analysis of maternal and miscarriage DNA for single nucleotide polymorphism (SNP) markers were used to simultaneously monitor maternal cell contamination (MCC), chromosomal status, and sex of the miscarriage tissue. NGS detection results of 21 chromosome abnormalities were consisted with that in karyotyping examination. These chromosome abnormalities samples included 9 chromosome 16 trisomies, 3 chromosome 22 trisomies, 2 chromosome 7 trisomies, 2 chromosome 18 trisomies, 1 chromosome 4 trisomies, one chromosome 10 trisomies, 1 chromosome 13 trisomies, 1 chromosome 15 trisomies and 1 sex chromosomal aneuploidies (45, X). Meanwhile, NGS analysis of 7 chromosome normalities was adapted to the karyotyping examination. The authors concluded that the findings of this study suggested that NGS combined with multiplex PCR is a viable alternative to karyotyping for detecting chromosomal abnormalities associated with miscarriage providing a novel strategy to rapid test the chromosome composition in the POC sample.
Tamura and co-workers (2021) stated that chromosomal abnormalities are a major cause of SA, and conventional G-banding is mainly used for chromosomal analysis. Recently, NGS has been introduced for chromosomal analysis. These investigators examined the applicability and use of NGS-based chromosomal analysis of POC on chorionic villus samples from SA. The results of chromosomal analysis of 7 chorionic villus samples from SA were compared between conventional G-banding and NGS-based chromosomal copy number analysis. Age dependency and frequency of each chromosomal aneuploidy were evaluated for 279 cases analyzed by NGS. Excluding 2 cases (culture failure and MCC), the results were consistent between G-banding and NGS. For cases analyzed by NGS, the rate of chromosomal abnormality increased in a maternal age-dependent manner. The frequency of each chromosomal aneuploidy detected by NGS was almost the same as that previously reported. Finally, NGS analysis was possible for difficult cases by G-banding analysis, such as culture failure, MCC, long-term storage cases, and low cell number. The authors concluded that chromosome analysis using NGS obtained comparable results to conventional G-banding and could detect segmental aneuploidy or mosaicism. These researchers stated that NGS-based analysis does not require cell culture or large cell number and is able to examine the difficult case to analyze by conventional G-banding; these findings suggested that chromosome analysis using NGS is a useful method for accurate and efficient POC testing in spontaneous abortion.

Furthermore, UpToDate reviews on “Recurrent pregnancy loss: Evaluation” (Tulandi and Al-Fozan, 2021), “Pregnancy loss (miscarriage): Assessment, counseling, and choice of treatment” (Prager et al, 2021a) and “Pregnancy loss (miscarriage): Risk factors, etiology, clinical manifestations, and diagnostic evaluation” (Prager et al, 2021b) do not mention next generation sequencing as a management tool.
### Medically necessary tests:

CPT codes covered if selection criteria are met:

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>58100</td>
<td>Endometrial sampling (biopsy) with or without endocervical sampling (biopsy), without cervical dilation, any method (separate procedure)</td>
</tr>
<tr>
<td>58340</td>
<td>Catheterization and introduction of saline or contrast material for saline infusion sonohysterography (SIS) or hysterosalpingography</td>
</tr>
<tr>
<td>58555 - 58563</td>
<td>Hysteroscopy, diagnostic or surgical</td>
</tr>
<tr>
<td>74740</td>
<td>Hysterosalpingography, radiological supervision and interpretation</td>
</tr>
<tr>
<td>76831</td>
<td>Saline infusion sonohysterography (SIS), including color flow Doppler, when performed</td>
</tr>
<tr>
<td>76856 - 76857</td>
<td>Ultrasound, pelvic (non-obstetric)</td>
</tr>
<tr>
<td>81241</td>
<td>F5 (coagulation factor V) (e.g., hereditary hypercoagulability) gene analysis, Leiden variant</td>
</tr>
<tr>
<td>81402 - 81408</td>
<td>Molecular pathology [not covered for mitochondrial DNA variations analysis]</td>
</tr>
<tr>
<td>83890 - 83914</td>
<td>Molecular diagnostics [not covered for preimplantation genetic screening]</td>
</tr>
<tr>
<td>84443</td>
<td>Thyroid stimulating hormone (TSH)</td>
</tr>
<tr>
<td>85307</td>
<td>Activated Protein C (APC) resistance assay</td>
</tr>
<tr>
<td>85335</td>
<td>Factor inhibitor test</td>
</tr>
<tr>
<td>85337</td>
<td>Thrombomodulin</td>
</tr>
<tr>
<td>85705</td>
<td>Thromboplastin inhibition, tissue</td>
</tr>
<tr>
<td>86146</td>
<td>Beta 2 Glycoprotein I antibody, each [IgG or IgM]</td>
</tr>
<tr>
<td>86147</td>
<td>Cardiolipin (phospholipid) antibody, each Ig class</td>
</tr>
<tr>
<td>86800</td>
<td>Thyroglobulin antibody</td>
</tr>
<tr>
<td>86828 - 86829</td>
<td>Antibody to human leukocyte antigens (HLA), solid phase assays (e.g., microspheres or beads, ELISA, flow cytometry); qualitative assessment of the presence or absence of antibody(ies) to HLA Class I and/or Class II HLA antigens</td>
</tr>
<tr>
<td>Code</td>
<td>Code Description</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>86830 - 83831</td>
<td>Antibody to human leukocyte antigens (HLA), solid phase assays (e.g., microspheres or beads, ELISA, Flow cytometry); antibody identification by qualitative panel using complete HLA phenotypes, HLA Class I or HLA Class II</td>
</tr>
<tr>
<td>86832 - 86833</td>
<td>Antibody to human leukocyte antigens (HLA), solid phase assays (e.g., microspheres or beads, ELISA, Flow cytometry); high definition qualitative panel for identification of antibody specificities (e.g., individual antigen per bead methodology), HLA Class I or HLA II</td>
</tr>
<tr>
<td>86834 - 86835</td>
<td>Antibody to human leukocyte antigens (HLA), solid phase assays (e.g., microspheres or beads, ELISA, Flow cytometry); semi-quantitative panel (e.g., titer), HLA Class I or HLA Class II</td>
</tr>
<tr>
<td>88230 - 88239</td>
<td>Tissue culture</td>
</tr>
<tr>
<td>88245 - 88269</td>
<td>Chromosome analysis for breakage syndromes [not covered for preimplantation genetic screening]</td>
</tr>
<tr>
<td>88271 - 88275</td>
<td>Molecular cytogenetics [not covered for preimplantation genetic screening]</td>
</tr>
<tr>
<td>88280 - 88289</td>
<td>Chromosome analysis (additional karyotypes, specialized banding techniques, cells counted, high resolution study) [not covered for preimplantation genetic screening]</td>
</tr>
<tr>
<td>88291</td>
<td>Cytogenetics and molecular cytogenetics, interpretation and report [not covered for preimplantation genetic screening]</td>
</tr>
<tr>
<td>89325</td>
<td>Sperm antibodies</td>
</tr>
<tr>
<td>Modifier 3G</td>
<td>F2, commonly called prothrombin (20210, others) (hypercoagulable state) prothrombin (factor II, 20210A) (hypercoagulable state) [not covered for preimplantation genetic screening]</td>
</tr>
</tbody>
</table>

HCPCS not covered for indications listed in the CPB:

<p>| J1645 | Injection, dalteparin sodium, per 2500 IU [not covered for recurrent pregnancy loss and autoantibodies or coagulation abnormality] |
| J1650 | Injection, enoxaparin sodium, 10 mg [not covered for recurrent pregnancy loss and autoantibodies or coagulation abnormality] |</p>
<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>J1652</td>
<td>Injection, fondaparinux sodium, 0.5 mg [not covered for recurrent pregnancy loss and autoantibodies or coagulation abnormality]</td>
</tr>
<tr>
<td>J1655</td>
<td>Injection, tinzaparin sodium, 1000 IU [not covered for recurrent pregnancy loss and autoantibodies or coagulation abnormality]</td>
</tr>
<tr>
<td></td>
<td>Other HCPCS related to the CPB:</td>
</tr>
<tr>
<td>J0135</td>
<td>Injection, adalimumab, 20 mg</td>
</tr>
<tr>
<td>J0717</td>
<td>Injection, certolizumab pegol, 1 mg (code may be used for Medicare when drug administered under the direct supervision of a physician, not for use when drug is self-administered)</td>
</tr>
<tr>
<td>J1438</td>
<td>Injection, etanercept, 25 mg (code may be used for Medicare when drug administered under the direct supervision of a physician, not for use when drug is self-administered)</td>
</tr>
<tr>
<td>J1602</td>
<td>Injection, golimumab, 1 mg, for intravenous use</td>
</tr>
<tr>
<td>J1745</td>
<td>Injection, infliximab, excludes biosimilar, 10 mg</td>
</tr>
<tr>
<td>J3358</td>
<td>Ustekinumab, for intravenous injection, 1 mg</td>
</tr>
<tr>
<td>S3800 - S3870</td>
<td>Genetic testing/gene sequence analysis</td>
</tr>
<tr>
<td>S9359</td>
<td>Home infusion therapy, antitumor necrosis factor intravenous therapy (e.g., Infliximab); administrative services professional pharmacy series, care coordination, and all necessary supplies and equipment (drugs and nursing visits coded separately)</td>
</tr>
<tr>
<td></td>
<td>ICD-10 codes covered if selection criteria are met [for medically necessary tests]:</td>
</tr>
<tr>
<td>N96</td>
<td>Recurrent pregnancy loss</td>
</tr>
<tr>
<td>O09.291 - O09.299</td>
<td>Supervision of pregnancy with other poor reproductive or obstetrical history</td>
</tr>
<tr>
<td>O26.20 - O26.23</td>
<td>Pregnancy care for patient with recurrent pregnancy loss</td>
</tr>
<tr>
<td>Z31.441</td>
<td>Encounter for testing of male partner of patient with recurrent pregnancy loss</td>
</tr>
</tbody>
</table>

*Experimental and investigational tests and treatments:*

CPT codes not covered for indications listed in the CPB:
<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>38242</td>
<td>Allogeneic lymphocyte infusions</td>
</tr>
<tr>
<td>81228</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (eg, bacterial artificial chromosome [BAC] or oligo-based comparative genomic hybridization [CGH] microarray analysis)</td>
</tr>
<tr>
<td>81229</td>
<td>Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities</td>
</tr>
<tr>
<td>81240</td>
<td>F2 (prothrombin, coagulation factor II) (eg, hereditary hypercoagulability) gene analysis, 20210G&gt;A variant</td>
</tr>
<tr>
<td>81291</td>
<td>MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)</td>
</tr>
<tr>
<td>81400</td>
<td>Molecular pathology procedure, Level 1 (eg, identification of single germline variant [eg, SNP] by techniques such as restriction enzyme digestion or melt curve analysis) [angiotensin converting enzyme (ACE) gene polymorphisms testing], [plasminogen activator inhibitor-1 (PAI-1) gene polymorphisms testing] [interleukin-18 gene polymorphisms testing]</td>
</tr>
<tr>
<td>81401</td>
<td>Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)</td>
</tr>
<tr>
<td>81415-81417</td>
<td>Exome (eg, unexplained constitutional or heritable disorder or syndrome)</td>
</tr>
<tr>
<td>Code</td>
<td>Code Description</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>81460</td>
<td>Whole mitochondrial genome (e.g., Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS], myoclonic epilepsy with ragged-red fibers [MERFF], neuropathy, ataxia, and retinitis pigmentosa [NARP], Leber hereditary optic neuropathy [LHON]), genomic sequence, must include sequence analysis of entire mitochondrial genome with heteroplasmy detection</td>
</tr>
<tr>
<td>82397</td>
<td>Chemiluminescent assay [inhibin B]</td>
</tr>
<tr>
<td>83090</td>
<td>Homocysteine</td>
</tr>
<tr>
<td>83516</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method [serum anti-heat shock protein antibodies (e.g., anti-HSP60 and anti-HSP70) levels]</td>
</tr>
<tr>
<td>83520</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified [cytokine polymorphisms analysis (Th1/Th2 intra-cellular cytokine ratio) [Inhibin B] [antiphosphatidylinositol and antiphosphatidyglycerol antibodies] [antiphosphatidic acid antibodies] [tumor necrosis factor alpha gene polymorphisms testing]</td>
</tr>
<tr>
<td>85300</td>
<td>Clotting inhibitors or anticoagulants; antithrombin III, activity</td>
</tr>
<tr>
<td>85301</td>
<td>Clotting inhibitors or anticoagulants; antithrombin III, antigen assay</td>
</tr>
<tr>
<td>85302 - 85306</td>
<td>Clotting inhibitors or anticoagulants; protein C and protein S</td>
</tr>
<tr>
<td>85415</td>
<td>Fibrinolytic factors and inhibitors; plasminogen activator</td>
</tr>
<tr>
<td>86021</td>
<td>Antibody identification; leukocyte antibodies</td>
</tr>
<tr>
<td>86148</td>
<td>Ant-phosphatidylserine (phospholipid) antibody</td>
</tr>
<tr>
<td>86255</td>
<td>Fluorescent noninfectious agent antibody; screen, each antibody [antiadrenal antibodies]</td>
</tr>
<tr>
<td>86256</td>
<td>titer, each antibody</td>
</tr>
<tr>
<td>86353</td>
<td>Lymphocyte transformation, mitogen (phytomitogen) or antigen induced blastogenesis [mixed lymphocyte culture reactions]</td>
</tr>
<tr>
<td>86357</td>
<td>Natural killer (NK) cells, total count</td>
</tr>
<tr>
<td>Code</td>
<td>Code Description</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>86805</td>
<td>Lymphocytotoxicity assay, visual crossmatch; with titration</td>
</tr>
<tr>
<td>86807</td>
<td>Serum screening for cytotoxic percent reactive antibody (PRA); standard method</td>
</tr>
<tr>
<td>86812 - 86817</td>
<td>HLA typing</td>
</tr>
<tr>
<td>86825</td>
<td>Human leukocyte antigen (HLA) crossmatch, non-cytotoxic (eg, using flow cytometry); first serum sample or dilution</td>
</tr>
<tr>
<td>86826</td>
<td>each additional serum sample or sample dilution</td>
</tr>
<tr>
<td>86950</td>
<td>Leukocyte transfusion</td>
</tr>
<tr>
<td>88142</td>
<td>Cytopathology, cervical or vaginal (any reporting system), collected in preservative fluid, automated thin layer preparation; manual screening under physician supervision [reproductive immunophenotype CD3+, CD4+, CD5+, CD8+, CD16+, CD19+, CD56+]</td>
</tr>
<tr>
<td>88184</td>
<td>Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker [NK activity]</td>
</tr>
<tr>
<td>88185</td>
<td>each additional marker (List separately in addition to code for first marker) [NK activity]</td>
</tr>
<tr>
<td>88189</td>
<td>Flow cytometry, cell cycle or DNA analysis [reproductive immunophenotype CD3+, CD4+, CD5+, CD8+, CD16+, CD19+, CD56+]</td>
</tr>
<tr>
<td>88360</td>
<td>Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual</td>
</tr>
<tr>
<td>88360</td>
<td>using computer-assisted technology</td>
</tr>
<tr>
<td>89290 - 89291</td>
<td>Biopsy, oocyte polar body or embryo blastomere, microtechnique (for pre-implantation genetic diagnosis); less than, equal to, or greater than 5 embryos [for recurrent pregnancy loss]</td>
</tr>
<tr>
<td>90283</td>
<td>Immune globulin (IgIV), human, for intravenous use</td>
</tr>
</tbody>
</table>

ICD-10 codes not covered for indications listed in the CPB [for experimental and investigational tests and treatments]:
### Recurrent Pregnancy Loss

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>N94.89</td>
<td>Other and unspecified conditions associated with female genital organs and menstrual cycle</td>
</tr>
<tr>
<td>N94.9</td>
<td></td>
</tr>
<tr>
<td>N96</td>
<td>Recurrent pregnancy loss</td>
</tr>
<tr>
<td>O03.0 - O03.9</td>
<td>Spontaneous abortion</td>
</tr>
<tr>
<td>O09.211 - O09.219</td>
<td>Supervision of pregnancy with history of pre-term labor</td>
</tr>
<tr>
<td>O09.291 - O09.299</td>
<td>Supervision of pregnancy with other poor reproductive or obstetrical history</td>
</tr>
<tr>
<td>O26.20 - O26.23</td>
<td>Pregnancy care for patient with recurrent pregnancy loss</td>
</tr>
<tr>
<td>Z31.430</td>
<td>Encounter of female for testing for genetic disease carrier status for procreative management</td>
</tr>
<tr>
<td>Z31.438</td>
<td>Encounter for other genetic testing of female for procreative management</td>
</tr>
<tr>
<td>Z31.441</td>
<td>Encounter for testing of male partner of patient with recurrent pregnancy loss</td>
</tr>
<tr>
<td>Z31.5</td>
<td>Encounter for genetic counseling</td>
</tr>
<tr>
<td>Z87.51</td>
<td>Personal history of pre-term labor</td>
</tr>
</tbody>
</table>

The above policy is based on the following references:


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85. Prager S, Micks E, Dalton VK. Pregnancy loss (miscarriage): Assessment, counseling, and choice of treatment. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed June 2021a.

86. Prager S, Micks E, Dalton VK. Pregnancy loss (miscarriage): Risk factors, etiology, clinical manifestations, and diagnostic evaluation. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed June 2021b.


diagnosis the ideal embryo selection method in aneuploidy

95. Salimi E, Karimi-Zarchi M, Dastgheib SA, et al. Association of
promoter region polymorphisms of IL-6 and IL-18 genes with risk of
recurrent pregnancy loss: A systematic review and meta-analysis.

96. Sargent IL, Dokras A. Embryotoxicity as a marker for recurrent

97. Scott JR, Branch DW, Kochenour NK, Ward K. Intravenous
immunoglobulin treatment of pregnant patients with recurrent
pregnancy loss caused by antiphospholipid antibodies and Rh

98. Scott JR. Immunotherapy for recurrent miscarriage. Cochrane

99. Sierra S, Stephenson M. Genetics of recurrent pregnancy loss.

100. Singh A, Dantas ZN, Stone SC, Asch RH. Presence of thyroid
antibodies in early reproductive failure: Biochemical vs. clinical

101. Smith CI, Hammarstrom L. Intravenous immunoglobulin in

102. Stephenson M, Kutteh W. Evaluation and management of

immunoglobulin and idiopathic secondary recurrent miscarriage: A
multicentered randomized placebo-controlled trial. Hum Reprod.
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104. Su MT, Lin SH, Chen YC, Kuo PL. Genetic association studies of
ACE and PAI-1 genes in women with recurrent pregnancy loss: A
systematic review and meta-analysis. Thromb Haemost.

Phosphatidylserine-dependent antiprothrombin antibodies are not
useful markers for high-risk women with recurrent miscarriages.

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sperm DNA fragmentation and idiopathic recurrent pregnancy
loss: A systematic review and meta-analysis. Reprod Biomed

107. Takeshita T. Diagnosis and treatment of recurrent miscarriage


114. Tulandi T, Al-Fozan HM. Management of couples with recurrent pregnancy loss. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed January 2015b.


119. Wapner RJ, Cowchock FS, Shapiro SS. Successful treatment in two women with antiphospholipid antibodies and refractory pregnancy


AETNA BETTER HEALTH® OF PENNSYLVANIA

Amendment to
Aetna Clinical Policy Bulletin Number: 0348 Recurrent Pregnancy Loss

There are no amendments for Medicaid.

revised 09/10/2021