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Recurrent pregnancy loss

Rahat Khan, Vikram Talaulikar and Hassan Shehata

DEFINITION

Recurrent pregnancy loss (RPL) refers to the consecutive loss of three or more clinically recognized pregnancies prior to the 20th week of gestation (excluding ectopic, molar and biochemical pregnancies). RPL is classified into two categories: primary RPL, which consists of repeated miscarriages in which a pregnancy has never been carried to viability; and secondary RPL, in which a live birth has occurred at some time. Secondary RPL confers a better prognosis than primary RPL[1].

INCIDENCE

About 10–15% of all clinically recognized pregnancies end in miscarriage. Approximately 2% of women experience two and 0.4–1% of women experience three consecutive losses[2]. At less than 6 weeks' gestation the risk of miscarriage ranges from 22 to 57%, it declines to 15% at 6–10 weeks and 2–3% after 10 weeks of gestation[3].

RISK FACTORS AND ETIOLOGY

Couples with pregnancy loss usually express concern regarding the cause and risk of recurrence. The risk of miscarriage increases with maternal age and parity, being 19% at less than 35 years and increasing to 47% in those over 35 years. In a similar fashion, the risk of miscarriage increases from 14–21% after one miscarriage to 24–29% after two and 31–33% after three pregnancy losses. The minimum diagnostic workup of couples experiencing RPL consists of a complete medical, surgical, genetic and family history and a physical examination (see below).

General causes of RPL are shown in Table 1.

GENETIC FACTORS

The highest rate of cytogenetically abnormal fetuses occurs earliest in gestation, with rates declining after the embryonic period (>30 mm crown–rump length (CRL)).

Table 1 General causes of recurrent pregnancy loss

Causes	*Percentage* (%)
Genetic factors – chromosomal abnormality	3–5
Primary miscarrier (no live births)	7
Secondary miscarrier (1 or more live births)	50
Anatomic causes	5–10
Immune mechanisms	50
Thrombophilias	10–13
Endocrine	20
Infection	1
Unexplained	15

Parental chromosomal abnormalities

In approximately 3–5% of couples with RPL, one of the partners carries a balanced structural chromosomal anomaly (versus 0.7% of the general population), the most common being balanced reciprocal (60%) and Robertsonian (40%) translocations.

Aneuploidy

The risk of aneuploidy (meiotic non-disjunction, polypoid from fertilization abnormalities) increases as the number of previous miscarriages increases.

Other

Progesterone receptor gene polymorphism may play a role in RPL and is an active area of investigation[4]. Maternal diseases including sickle cell anemia, myotonic dystrophy, Marfan's syndrome, homocystinuria, factor VIII deficiency, dysfibrinogenemia and Ehler's Danlos syndrome are all associated with increased fetal loss.

Investigations and treatment

Couples with a history of RPL should have peripheral blood karyotyping, and cytogenetic analysis of the products of conception should be performed if the next pregnancy fails.

Genetic counseling can provide the couple with a prognosis for future pregnancy, as well as offer familial chromosomal studies and appropriate preimplantation genetic diagnostic procedures in future pregnancies. In addition, the couple should be informed that they have a 40–50% chance of a healthy live birth in future untreated pregnancies following natural conception.

ANATOMIC CAUSES

Acquired and congenital uterine abnormalities are responsible for 10–15% of RPL[5] and may be associated with fetal growth restriction and preterm delivery.

Uterine anomalies

The most frequent uterine defects include septate, bicornuate and didelphic abnormalities. The septate uterus is most common and associated with the poorest reproductive outcome (miscarriage rate more than 60% in untreated cases)[6,7]. Other anatomic causes of RPL are diethylstilbestrol exposure related anomalies, Asherman's syndrome, leiomyomas and endometrial polyps. A primary endometrial receptor defect appears to be responsible for RPL in some patients.

Investigation and treatment

Transvaginal ultrasound is useful for making a diagnosis of uterine anomalies[8]. Hysteroscopy is usually reserved for patients in whom intrauterine pathology is suspected and operative hysteroscopy is necessary. Transvaginal ultrasound assessment of the cervix during pregnancy may be useful in predicting preterm birth in cases of suspected cervical weakness. Magnetic resonance imaging (MRI) is useful for distinguishing between a septate and bicornuate uterus[8].

Cervical incompetence

No satisfactory objective test is available for cervical incompetence, and diagnosis is usually made on the basis of a history of late miscarriages, preceded by spontaneous rupture of membranes and painless cervical dilatation.

Investigation and treatment

The Medical Research Council (MRC)/Royal College of Obstetricians and Gynaecologists (RCOG) trial of elective cervical cerclage reported a small decrease in preterm birth and delivery of very low birth weight babies, the benefit being most marked in women with three or more recurrent second trimester miscarriages[9]. However, no significant improvement in perinatal survival was present.

IMMUNE MECHANISMS

Both autoimmune and alloimmune mechanisms have been proposed as explanations for RPL.

Antiphospholipid syndrome

Antiphospholipid antibodies (aPL) are present in 15% of women with RPL and 33% of women with systemic lupus erythematosus (SLE)[10]. In women with RPL associated with untreated aPL, the live birth rate may be as low as 10%. Primary antiphospholipid syndrome (APS), which predominantly affects young women, refers to the association of aPL and adverse pregnancy outcome or vascular thrombosis. Adverse pregnancy outcomes include three or more consecutive miscarriages before 10 weeks' gestation; one or more morphologically normal fetal loss after 10 weeks' gestation; and one or more preterm birth before 34th week of gestation due to severe pre-eclampsia, eclampsia or placental insufficiency. When APS exists in chronic inflammatory disorders, such as SLE, it is referred to as secondary APS.

Investigation

To diagnose APS, it is mandatory that the patient have two positive tests at least 6 weeks apart for either lupus anticoagulant or anticardiolipin antibodies (aCL) of IgG and/or IgM class present in medium or high titers. In detection of lupus anticoagulant, the dilute Russell's viper venom (dRVVT) test is more sensitive and specific than the kaolin clotting time (KCT) or activated partial thromboplastin time (aPTT).

Treatment

Currently, several well controlled studies show that future live birth is significantly improved from 50% to 80% when a combination therapy of low dose aspirin (75 mg) plus heparin (5000 U once or twice a day) is prescribed. A recent randomized trial reported a high success rate with aspirin alone but included women with low titers of aPL only[11].

Antithyroid antibodies

Patients with treated thyroid dysfunction have no risk of increased miscarriage[12]. Although more women with RPL have antithyroid antibodies than in the general population, evidence that these antibodies actually cause pregnancy loss is lacking[13].

Investigation and treatment

Because several studies report an increased rate of fetal loss in women with high serum thyroid peroxidase (TPO) antibody concentrations, we propose that it should be investigated in women with RPL.

Current data suggest that in women with RPL and thyroid antibodies, treatment with L-thyroxine and/or prednisolone should be considered, although further controlled studies are essential[14].

Antinuclear antibodies

A connection exists between antinuclear antibodies (ANAs) and recurrent miscarriages, with a titer of over 1:40 causing concern.

Investigation and treatment

Measuring antinuclear and anti-dsDNA antibodies is not recommended as part of an evaluation of women with RPL.

Treatment with low dose prednisolone could be considered as a treatment modality in patients who have raised ANA, but further studies are needed.

Alloimmune factors

Allogeneic factors may cause RPL by a mechanism similar to that of graft rejection in transplant recipients. Human leukocyte antigen (HLA) sharing is a condition in which the normal process that allows for creation of maternal blocking antibodies in pregnancy is decreased. However, no clear evidence as yet proves an association between RPL and HLA incompatibility between couples.

Cytokines and miscarriage

Thomas Wegmann first proposed the immunotrophic hypothesis suggesting that a successful allo-pregnancy was a T helper 2 (Th2) phenomenon and demonstrating a Th2 cell cytokine profile response in normal pregnancy[15,16]. Since then a number of human and animal studies[17–20] further confirm the Th2 cytokine predominance associated with a successful pregnancy, although some controversy exists[21,22]. The apparently harmful Th1 cytokines, which can activate natural killer (NK) cells into lymphokine-activated killer (LAK) cells, include tumor necrosis factor (TNF)-α, interleukin (IL)-2, interferon (IFN)-γ, IL-12 and IL-18; the main Th2 type cytokines include IL-3, granulocyte macrophage colony stimulating factor (GM-CSF), CSF-1, IL-10 and transforming growth factor (TGF)-β[23,24]. A study published in 2008 demonstrated that women with a history of unexplained recurrent failed *in vitro* fertilization (IVF) treatment not only have a Th1 bias but also that this polarization is enhanced following hormonal manipulations that accompany IVF treatment[25].

It is as yet unclear as to what should be the proportion of the Th1 cytokines at the fetomaternal surface to either damage or benefit any ongoing pregnancy[26–28].

Role of other markers/substrates

In a study measuring serum concentrations of macrophage inhibitory cytokine (MIC)-1 in asymptomatic women at 6–13 weeks' gestation who subsequently miscarried or who had already miscarried, MIC-1 concentrations were a third of those in women who had ongoing pregnancies, an observation which suggested a possible predictive as well as therapeutic potential for MIC-1[29]. Recurring miscarriages also have shown an association with elevated serum homocysteine concentrations in other studies[30,31].

Natural killer cells and miscarriage

NK cells comprise about 10–15% of peripheral blood lymphocytes. Two distinct subsets of human NK cells are possible, depending on the cell surface density of the CD56 molecule. Approximately 90% of peripheral blood human NK cells are CD56dim and express high levels of FcγIII (CD16) as well as perforin. In contrast, a minority (approximately 10%) of NK cells are CD56bright and CD16dim. These CD16dim cells are the primary source of NK cell derived cytokines and thought to

be an important regulatory subset[32,33]. In the uterus, the NK cells form the largest population of the leukocytes and are predominantly the CD56bright variety.

Studies suggest that uterine NK cell function in preimplantation endometrium is to promote angiogenesis, and thus provide a potential mechanism by which the increased endometrial uterine NK cell density causes miscarriage by the final common pathway of excessive oxidative stress[34,35].

NK cell receptor expression

An imbalance between inhibitory and activating receptor expression is present in women with implantation failures[36]. When compared with normal controls, CD158a and CD158b inhibitory receptor expression by CD56dim/CD16+ and CD56bright/CD16− NK cells was significantly decreased, and CD161 activating receptor expression by CD56+/CD3+ NK cells was significantly increased in women with implantation failures[35]. In another study, infertile women had a significantly higher expression of NK cell activation markers of the CD69+ type[37].

NK cell cytotoxicity

Aoki *et al*. reported increased preconceptional NK cell activity in women with unexplained RPL[38], while other studies revealed that infertile women have higher levels of activated NK cells compared with control multiparous women and that women with elevated levels of activated NK cells have a poorer IVF treatment and pregnancy outcome[39,40]. In summary, despite a few contradictory studies[41,42] a significant amount of data points to increased peripheral or local NK cell activity contributing towards the pathogenesis of recurrent miscarriage.

NK cell numbers

An abnormal increase in peripheral blood NK cell parameters (either in NK cell absolute values or in proportion (%) prior to conception or during early pregnancy) is associated with recurrent miscarriage and infertility with multiple implantation failures[40,43]. Data suggest that there may be a significant difference in subpopulations among uterine NK cells, with a greater proportion of cells being CD56dim, which may have important functional implications. Some studies using CD57 monoclonal antibodies (mAb) demonstrated elevated NK cell populations in decidua[44,45], whereas studies using CD56 failed to document change[46,47].

Investigation

Specific immunological testing should be conducted as a part of ongoing research in a specialized center. This includes NK cells (number and activation), MIC-1, Th1 and Th2 cytokines, HLA typing, mixed lymphocyte antibody tests and mixed lymphocyte culture reactions.

Treatment

In the absence of strong data to prove the immune-endocrine nature of abnormalities in recurrent miscarriage, most of the clinical therapies used over the years have been of an empiric nature.

Progesterone In a subgroup analysis of three trials involving 91 women with recurrent miscarriages, progestogen treatment significantly decreased the miscarriage rate compared with placebo or no treatment[48]. Despite this the current RCOG Guideline No. 17 (published in 2003) states that there is insufficient evidence to evaluate the effect of progesterone or human

chorionic gonadotropin supplementation in pregnancy to prevent a miscarriage[49].

Heparin Heparin, in addition to its anticoagulant effects, suppresses NK cell cytotoxicity and antagonizes IFN-γ action by inhibiting its binding to the cell surface[50].

Prednisolone therapy A recent study by Thum *et al*. demonstrated that prednisolone has a similar *in vitro* suppression effect on NK cell cytolytic capability to intravenous immunoglobulins (IV)[51]. In addition, Xu *et al*. showed that prednisolone had a suppressive effect on TNF-α (Th1 cytokine) production from placental tissue[44]. Furthermore, Quenby *et al*.[35] reported that prednisolone could suppress NK cell levels and reduce the miscarriage rate in women with a history of recurrent miscarriage.

IV immunoglobulins Women with a history of recurrent miscarriage have a higher level of NK cell cytotoxicity which can be suppressed by co-culture of the NK cells with immunoglobulin-G (IVIg)[45]. However, women who have elevated NK cell cytotoxicity and a history of recurrent miscarriage or recurrent failed implantation during IVF may have a better obstetric outcome if they have IVIg infusion during IVF treatment or early pregnancy[52,53].

TNF-α inhibitors, sildenafil and 1,25-dihydroxyvitamin D3 Winger *et al*.[54] used mAb directed against TNF-α along with IVIg to improve pregnancy rates in their IVF patients. Concerns about such use, however, relate in part to an increased risk of infectious diseases, especially tuberculosis.

Evans *et al*.[55] demonstrated that several components of vitamin D metabolism and signaling are strongly expressed in human uterine decidua from first trimester pregnancies, suggesting that locally produced 1,25-dihydroxyvitamin D3 may exert immunosuppressive effects during early stages of gestation.

A study in 2008 by Jerzak *et al*.[56], evaluating the effects of vaginal sildenafil on NK cell activity, suggested that NK cell activity was significantly decreased after vaginal sildenafil therapy in the study women.

THROMBOPHILIAS AND FIBRINOLYTIC FACTORS

Retrospective studies have suggested an association between inherited thrombophilic defects, fetal loss and late pregnancy complications, with a presumed mechanism being defective placentation and microthrombi in the placental vasculature. Inherited thrombophilias include factor V Leiden, protein C and S deficiency, antithrombin III deficiency, activated prothrombin C resistance (APCR), methylene tetrahydrofolate reductase (MTHFR) C677T and G20210A prothrombin gene mutation. Acquired thrombophilia includes anticardiolipin antibodies and lupus anticoagulant.

In the absence of treatment, factor V Leiden is associated with an increased risk of miscarriage, compared with a normal factor V genotype. Factor V Leiden is carried by 5% of Caucasians, but is rarely found among Blacks. Other inherited thrombophilias are rare, and no conclusive studies have been conducted to prove their causality in RPL. Moreover, RPL has no significant association with plasminogen activator inhibitor-I4G/5G polymorphism or increased plasminogen activator inhibitor activity[57]. Procoagulant microparticles were shown to be associated with early and late unexplained pregnancy loss in one pilot study[58].

Investigations and treatment

A full inherited and acquired thrombophilia screen is recommended in women with RPL.

The general approach is to treat women with thrombophilia with a combination of low dose

aspirin and low molecular weight heparin. Therapy may need to be started before pregnancy occurs and continued to 6 weeks after birth (see also Chapter 9).

ENDOCRINE

Endocrine factors may be responsible for 15–20% of RPL.

Polycystic ovarian syndrome

Women with polycystic ovarian syndrome (PCOS) have a miscarriage rate of 20–40% as compared to the general obstetric population (10–20%). This may be related to elevated serum luteinizing hormone (LH) levels, high testosterone and androstenedione concentrations, or insulin resistance[59].

Investigation and treatment

Day 2–5 follicle stimulating hormone (FSH), LH, prolactin, sex hormone binding globulin, prolactin and transvaginal ultrasound are the recommended investigations in women with recurrent miscarriages.

Pre-pregnancy suppression of high LH by either clomiphene or metformin among ovulatory women with RPL and PCOS does not improve the live birth rate.

Luteal phase defect

It is controversial as to whether such a defect exists and whether it is related to miscarriage.

Investigation and treatment

Diagnosis of luteal phase defect based on endometrial biopsy is not predictive of fertility status, and single or multiple progesterone levels are not predictive of future pregnancy outcome[60].

Treatment with progesterone supplementation does not have a beneficial effect on pregnancy outcome.

Diabetes

Diabetic gravida with hemoglobin A1c levels in the first trimester of more than 8 are at increased risk of miscarriage and fetal malformations.

Investigation and treatment

Routine screening for diabetes with the oral glucose tolerance test in asymptomatic women with RPL should not be performed unless a random glucose value is elevated.

Diabetic women with RPL should be treated in a multidisciplinary joint diabetic clinic.

Hyperprolactinemia

Normal circulating levels of prolactin may play an important role in maintaining pregnancy.

Investigation and treatment

A study of 64 hyperprolactinemic women with RPL randomly assigned subjects to therapy with bromocriptine or no therapy[61]. Treatment to lower prolactin concentrations was associated with a higher rate of successful pregnancy (86% versus 52%). Prolactin levels during early pregnancy were significantly greater in women who miscarried[61].

Thyroid disease

Poorly controlled thyroid disease (hypo- or hyperthyroidism) is associated with infertility

and pregnancy loss. Excess thyroid hormone increases the risk of miscarriage[62].

Investigation and treatment

Routine screening for abnormal thyroid function tests should not be performed in asymptomatic women. Women with overt thyroid disease should be referred to a specialist.

INFECTION

Some infections, including listeriosis, toxoplasmosis, cytomegalovirus and primary genital herpes, cause sporadic pregnancy loss, but no infectious agent has been proven to cause RPL[63].

Investigation and treatment

Routine cervical cultures for *Chlamydia* or *Mycoplasma*, vaginal evaluation for bacterial vaginosis and TORCH (toxoplasma, rubella, cytomegalovirus and herpes simplex) serology are not useful in the evaluation of RPL, but they may be indicated by patient history.

Screening for and treatment of bacterial vaginosis in early pregnancy in women with a history of second trimester miscarriage or preterm labor may reduce the risk of RPL.

OTHER CAUSES

Chemicals

Chemicals which have been associated with RPL include nitrous oxide, arsenic, aniline dyes, benzene, ethylene oxide, lead, pesticides, mercury and cadmium.

Personal habits

The association between RPL and smoking, alcohol use or caffeine consumption is unclear[64].

Decreased ovarian reserve

Women with unexplained RPL have a higher incidence of elevated day 3 FSH and estradiol levels than women with known causes of RPL.

Day 1–3 FSH or a clomiphene challenge test can be considered in women of any age with RPL. A day 3 FSH level of less than 15 mIU/ml and high estradiol levels more than 80 pg/ml are associated with reduced oocyte numbers.

UNEXPLAINED

A significant proportion of cases of RPL (15%) remain unexplained, despite detailed investigations. These women can be reassured that the prognosis for a successful pregnancy outcome with supportive care alone is in the region of 75%. Treatment offered to couples with unexplained RPL includes the following:

- *Lifestyle modification* Weight loss, exercise, avoiding alcohol, caffeine intake and smoking.
- *Progesterone* Large randomized controlled studies demonstrating the efficacy of progesterone treatment are lacking, but the drug is widely prescribed to women with RPL.
- *IVF and preimplantation genetic diagnosis (PGD)* Studies evaluating the value of IVF in women with RPL have yielded mixed results. A combination of IVF and PGD at the 6–8 cell stage appears promising[65].
- *Oocyte donation* Ovum donation can overcome the problem of poor quality oocytes and has been associated with a live birth rate of 88% in women with RPL[66].
- *Combination therapy* A recent observational study compared 50 pregnant women treated before and during pregnancy with prednisolone (20 mg/day), progesterone (200 mg/day), aspirin (100 mg/day) and folate (5 mg/day) with 52 women who were

not treated; the first trimester pregnancy loss rate was 19% in the treated and 63% in the untreated group. Although this difference was not statistically significant, it is clinically important and perhaps resulted from insufficient study numbers[67].

- *Complementary therapies* Many acupuncturists report success in treating women with a history of RPL. Dietary supplementation with vitamin B complex, including folic acid and co-enzyme Q10 may suggest a reduction in RPL. Reflexology, a holistic therapy, attempts to relieve stress, pain and muscle tension and thus help to reduce miscarriages.

BIOCHEMICAL PREGNANCY LOSS

A biochemical or pre-clinical pregnancy loss is defined as loss of a biochemically evident pregnancy before it is identifiable on ultrasound.

Early pregnancy loss occurs in 75% of all pregnancies, out of which 15–20% are clinically recognized. However, the true rate of early pregnancy loss is close to 50%, because of the high number of chemical pregnancies that are not recognized in the 2–4 weeks after conception. In a classic study by Wilcox *et al*. in 1988[68], 221 women were followed up during 707 total menstrual cycles. A total of 198 pregnancies were recorded; 43 (22%) were lost before onset of menses and another 20 (10%) were clinically recognized losses.

Investigations

No investigative studies have been conducted on the recurrent biochemical pregnancy loss.

A US study on 122 women experiencing IVF implantation failure with a negative pregnancy test and 20 women with chemical pregnancy loss evaluated aPL, ANA and elevated NK cells[69]. Women with chemical pregnancies had a higher frequency of aPL than women with implantation failure associated with a negative pregnancy test. The prevalence of ANA and NK cells did not differ between the two groups. The authors concluded that the mechanisms involved in chemical pregnancies may be the result of defective angiogenesis as compared to pregnancies with a negative pregnancy test which involve implantation failure.

Treatment

As not much work has been done on the diagnosis and treatment modalities of chemical pregnancies, it is a very challenging area of reproductive medicine. A short trial of low dose prednisolone could be the way forward in the treatment of recurrent miscarriage, especially as the safety of prednisolone is well established. High quality data on management of biochemical RPL are limited and, therefore, therapeutic intervention is largely guided by the underlying cause.

CONCLUSION

RPL is an emotionally traumatic experience for a couple. Multidisciplinary teams expert in managing patients with RPL should coordinate evaluation and management. These should include gynecologists, geneticists, rheumatologists, hematologists, immunologists and reproductive specialists. High quality data on management of RPL are limited; therapeutic intervention is largely guided by the underlying cause of RPL. In all cases, emotional support is important in caring for these anxious couples.

REFERENCES

1. Paukku M, Tulppala M, Puolakkainen M, *et al*. Lack of association between serum antibodies to Chlamydia trachomatis and a his-

tory of recurrent pregnancy loss. *Fertil Steril* 1999;72:427
2. Salat-Baroux J. Recurrent spontaneous abortions. *Reprod Nutr Dev* 1988;28:1555
3. Edmonds DK, Lindsay KS, Miller JF, *et al*. Early embryonic mortality in women. *Fertil Steril* 1982;38:447
4. Li TC, Tuckerman EM, Laird SM. Endometrial factors in recurrent miscarriage. *Hum Reprod Update* 2002;8:43
5. Hill JA. Recurrent pregnancy loss. In: Ryan KJ, Berkowitz RS, Barbieri RL, Dunaif A, eds. *Kistner's Gynaecology and Women's Health*, 7th edn. St Louis: Mosby,1999:396
6. Homer HA, Li TC, Cooke LD. The septate uterus: A review of management and reproductive outcome. *Fertil Steril* 2000;73:1
7. Proctor JA, Haney AF. Recurrent first trimester pregnancy loss is associated with uterine septum but not bicornuate uterus. *Fertil Steril* 2003;80:1212
8. Goldberg JM, Falcone T, Attaran M. Sonohysterographic evaluation of uterine abnormalities noted on hysterosalpingography. *Hum Reprod* 1997;12:2151–3
9. MRC/RCOG Working Party on Cervical Cerclage. Final report of the Medical Research Council/Royal College of Obstetricians and Gynaecologists multicentre randomised trial of cervical cerclage. *Br J Obstet Gynaecol* 1993;100:516–23
10. Reindollar RH. Contemporary issues for spontaneous abortion. Does recurrent abortion exist? *Obstet Gynecol Clin North Am* 2000;27:541–54
11. Empson M, Lassere M, Craig JC, Scott JR. Recurrent pregnancy loss with antiphospholipid antibody; a systematic review of therapeutic trials(1). *Obstet Gynecol* 2002;99:135
12. Glinoer D, Soto MF, Bourdoux P, *et al*. Pregnancy in patients with mild thyroid abnormalities; maternal and neonatal repercussions. *J Clin Endocrinol Metab* 1991;73:421–7
13. Satagnaro- Green A, Glinoer D. Thyroid autoimmunity and the risk of miscarriage. *Best Pract Res Clin Endocrinol Metab* 2004;18:167–81
14. Negro R, Formoso G, Mangieri T, *et al*. Levothyroxine treatment in euthyroid pregnant women with autoimmune thyroid disease: effects on obstetrical complications. *J Clin Endocrinol Metab* 2006;91:2587
15. Wegmann TG. Fetal protection against abortion: is it immuno-suppression or immunostimulation? *Ann Immunol (Inst Pasteur)* 1984;135:309–12
16. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a TH 2 phenomenon? *Immunol Today* 1993;14:353–6
17. Tangri S, Raghupathy R. Expression of cytokines in placentas of mice undergoing immunologically mediated spontaneous fetal resorptions. *Biol Reprod* 1993;49:850–6
18. Tangri S, Wegmann TG, Lin H, Raghupathy R. Maternal anti-placental reactivity in natural, immunologically mediated spontaneous fetal resorptions. *J Immunol* 1994;152:4903–11
19. Chaouat G, Menu E, Assal-Meliani A, *et al*. *Allopregnancy is a TH 2 like phenomenon: IL 10 can prevent CBA×DBA/2 fetal wastage and TH 2 cytokine can be induced in vivo by tau interferons*. Annecy, France: Colloque Foundation Marcel Merieux, 1994
20. Hill JA, Polgar K, Anderson DJ. T helper 1 type immunity to trophoblast in women with recurrent spontaneous abortion. *JAMA* 1995;273:1933–6
21. Rein DT, Schondorf T, Gohring UJ, *et al*. Cytokine expression in peripheral blood lymphocytes indicates a switch to T (HELPER) cells in patients with preeclampsia. *J Reprod Immunol* 2002;54:133–42
22. Schust DJ, Hill JA. Correlation of serum cytokine and adhesion molecule determinations with pregnancy outcome. *J Soc Gynecol Invest* 1996;3:259–61
23. King A, Hiby SE, Gardner L, *et al*. Recognition of trophoblast HLA class I molecules by decidual NK cell receptors – a review. *Placenta* 2000;21(Suppl A):S81–5
24. Raghupathy R, Maksheed M, Azizieh F, Al-Azemi MMK, Hassan NA, Bandar A. Th1 and Th2 cytokine profiles in successful pregnancy and unexplained recurrent abortions. In: Gupta SK, ed. *Reproductive Immunology 2000*. Dehli: Kluwer Academic Publisher, 2000:149–66
25. Kalu E, Bhaskaran S, Thum MY, *et al*. Serial estimation of Th1:Th2 cytokines profile in women

undergoing in-vitro fertilization embryo transfer. *Am J Reprod Immunol* 2008;59:206–11
26. Loke YW, King A. Current topic: interferon and human placental development. *Placenta* 1990;11:291–9
27. Ashkar AA, Croy BA. Functions of uterine natural killer cells are mediated by interferon gamma production during murine pregnancy. *Semin Immunol* 2001; 3:235–41
28. Yeaman GR, Collins JE, Currie JK, Guyre PM, Wira CR, Fanger MW. IFN-gamma is produced by polimorphonuclear neutrophils in human uterine endometrium and by cultured peripheral blood polimorphonuclear neutrophils. *J Immunol* 1998;160:5145–53
29. Tong S, Marjono B, Brown DA, *et al.* Serum concentrations of macrophage inhibitory cytokine 1 (MIC 1) as a predictor of miscarriage. *Lancet* 2004;363:129–30
30. Sikora J, Magnucki J, Zietek J, *et al.* Homocysteine serum concentration and uterine artery color Doppler examination in cases of recurrent miscarriages with unexplained etiology. *Neurol Endocrinol Lett* 2007;28:502–6
31. Sikora J, Magnucki J, Zietek J, *et al.* Homocysteine, folic acid and vitamin B12 concentration in patients with recurrent miscarriages. *Neurol Endocrinol Lett* 2007;28:507–12
32. Orange JS, Ballas ZK. Natural killer cells in human health and disease. *Clin Immunol* 2006;118:1–10
33. Cooper MA, Fehniger TA, Turner SC, *et al.* Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. *Blood* 2001;97:3146–51
34. Quenby S, Nik H, Innes B, *et al.* Uterine natural killer cells and angiogenesis in recurrent reproductive failure. *Hum Reprod* 2009;24:45–54
35. Quenby S, Kalumbi C, Bates M, Farquharson RG, Vince G. Prednisolone reduces preconceptual endometrial natural killer cells in women with recurrent miscarriage. *Fertil Steril* 2005;84:980–4
36. Ntrivalas EI, Bowser CR, Kwak-Kim J, Beaman KD, Gilman-Sachs A. Expression of killer immunoglobulin-like receptors on peripheral blood NK cell subsets of women with recurrent spontaneous abortions or implantation failures. *Am J Reprod Immunol* 2005;53:215–21
37. Coulam CB, Roussev RG. Correlation of NK cell activation and inhibition markers with NK cytotoxicity among women experiencing immunologic implantation failure after in vitro fertilization and embryo transfer. *J Assist Reprod Genet* 2003;20:58–62
38. Aoki K, Kajiura S, Matsumoto Y, *et al.* Preconceptional natural-killer-cell activity as a predictor of miscarriage. *Lancet* 1995;345:1340–2
39. Thum MY, Bhaskaran S, Abdalla HI, *et al.* An increase in the absolute count of CD56dimCD16+ CD69+ NK cells in the peripheral blood is associated with a poorer IVF treatment and pregnancy outcome. *Hum Reprod* 2004;19:2395–400
40. Fukui A, Fujii S, Yamaguchi E, Kimura H, Sato S, Saito Y. Natural killer cell subpopulations and cytotoxicity for infertile patients undergoing in vitro fertilisation. *Am J Reprod Immunol* 1999;41:413–22
41. Emmer PM, Nelen WL, Steegers EA, Hendriks JC, Veerhoek M, Joosten I. Peripheral natural killer cytotoxicity and CD56(pos)CD16(pos) cells increase during early pregnancy in women with a history of recurrent spontaneous abortion. *Hum Reprod* 2000;15:1163–9
42. Souza SS, Ferriani RA, Santos CM, Voltarelli JC. Immunological evaluation of patients with recurrent abortion. *J Reprod Immunol* 2002;56:111–21
43. Perricone R, Di Muzio G, Perricone C, *et al*. Fontana L, De Carolis C. High levels of peripheral blood NK cells in women suffering from recurrent spontaneous abortion are reverted from high-dose intravenous immunoglobulins. *Am J Reprod Immunol* 2006;55:232–9
44. Xu B, Makris A, Thornton C, Hennessy A. Glucocorticoids inhibit placental cytokines from cultured normal and preeclamptic placental explants. *Placenta* 2005;26:654–60
45. Ruiz JE, Kwak JY, Baum L, *et al.* Effect of intravenous immunoglobulin G on natural killer cell cytotoxicity in vitro in women with recurrent spontaneous abortion. *J Reprod Immunol* 1996;31:125–41
46. Quack KC, Vassiliadou N, Pudney J, Anderson DJ, Hill JA. Leukocyte activation in the decidua of chromosomally normal and abnormal fetuses from women with recurrent abortion. *Hum Reprod* 2001;16:949–55

47. Hill JA, Melling GC, Johnson PM. Immunohistochemical studies of human uteroplacental tissues from first-trimester spontaneous abortion. *Am J Obstet Gynecol* 1995;173:90–6
48. Szekeres-Bartho J, Balasch J. Progestagen therapy for recurrent miscarriage. *Hum Reprod Update* 2008;14:27–35
49. RCOG. *The Investigation and Treatment of Couples with Recurrent Miscarriage*. Guideline No. 17. London, UK: RCOG, Revised May 2003
50. Dosiou C, Giudice LC. NK cells in pregnancy and RPL: endocrine and immunological perspectives. *Endocr Rev* 2005;26:44–62
51. Thum M-Y, Bhaskaran S, Abdalla HI, Ford B, Sumar N, Bansal A. Prednisolone suppresses NK cell cytotoxicity in vitro in women with a history of infertility and elevated NK cell cytotoxicity. *Am J Reprod Immunol* 2008;59:259–65
52. Coulam CB, Goodman C. Increased pregnancy rates after IVF/ET with intravenous immunoglobulin treatment in women with elevated circulating C56+ cells. *Early Pregnancy* 2000;4:90–8
53. Stricker RB, Steinleitner A, Bookoff CN, Weckstein LN, Winger EE. Successful treatment of immunologic abortion with low-dose intravenous immunoglobulin. *Fertil Steril* 2000;73:536–40
54. Winger EE, Reed JL, Sherif A, *et al*. Treatment with tumor necrosis factor inhibitors and intravenous immunoglobulin improves live birth rates in women with recurrent spontaneous abortion. *Am J Reprod Immunol* 2008;60:8–16
55. Evans KN, Nguyen L, Chan J, *et al*. Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells. *Biol Reprod* 2006;75:816–22
56. Jerzak M, Kniotek M, Mrozek J, *et al*. Sildenafil citrate decreased natural killer cell activity and enhanced chance of successful pregnancy in women with a history of recurrent miscarriage. *Fertil Steril* 2008;90:1848–53
57. Sotiriadis A, Makrigiannakis A, Stefos T, *et al*. Fibrinolytic defects and recurrent miscarriage: a systematic review and meta-analysis. *Obstet Gynecol* 2007;109:1146
58. Laude I, Rongieres-Bertrand C, Boyer-Neumann C, *et al*. Circulating procoagulant microparticles in women with unexplained pregnancy loss: a new insight. *Thromb Haemostat* 2001;85:18
59. Okon MA, Laird SM, Tuckerman EM, Li TC. Serum androgen levels in women who have recurrent miscarriages and their correlation with markers of endometrial function. *Fertil Steril* 1998;69:682
60. Ogasawara M, Kajiura S, Kantano K, *et al*. Are serum progesterone levels predictive of recurrent miscarriage in future pregnancies. *Fertil Steril* 1997;68:806
61. Hirahara F, Andoh N, Sawai K, *et al*. Hyperprolactinemic recurrent miscarriage and result of randomized bromocriptine treatment trials. *Fertil Steril* 1998;70:246
62. Anselmo J, Cao D, Karrison T, *et al*. Fetal loss associated with excess thyroid hormone exposure. *JAMA* 2004;292:691
63. ACOG practice bulletin. Management of recurrent pregnancy loss. Number 24, February 2001. American College of Obstetricians and Gynecologists. *Int J Gynaecol Obstet* 2002;78:179
64. Christiansen OB, Nybo Anderson AM, Bosch E, *et al*. Evidence based investigations and treatment of recurrent pregnancy loss. *Fertil Steril* 2005;83:821–39
65. Egozcue J, Santalo J, Gimenez C, *et al*. Preimplantation genetic diagnosis. *Mol Cell Endocrinol* 2000;166:21
66. Trout SW, Seifer DB. Do women with unexplained recurrent pregnancy loss have higher day 3 serum FSH and estradiol values? *Fertil Steril* 2000;74:335
67. Tempfer CB, Kurz C, Bentz EK, *et al*. A combination treatment of prednisone, aspirin, folate, and progesterone in women with idiopathic recurrent miscarriage: a matched-pair study. *Fertil Steril* 2006;86:145–8
68. Wilcox AJ, Weinberg CR, O'Connor JF, *et al*. Incidence of early loss of pregnancy. *N Engl J Med* 1998;319:189–94
69. Roussev R, Coulam CB. Chemical pregnancies. *J Immunol Ultrasonograph Stud* 2003;48:323–8