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## Review

# Recurrent miscarriage: current concepts in diagnosis and treatment

Bettina Toth<sup>a,\*</sup>, Udo Jeschke<sup>c,2</sup>, Nina Rogenhofer<sup>b,1</sup>, Christoph Scholz<sup>c,2</sup>,  
Wolfgang Würfel<sup>d,3</sup>, Christian J. Thaler<sup>b,1</sup>, Antonis Makrigiannakis<sup>e,4</sup>

<sup>a</sup> Department of Gynecological Endocrinology and Fertility Disorders, Ruprecht-Karl University Heidelberg, Voßstr. 9, 69115 Heidelberg, Germany

<sup>b</sup> Department of Obstetrics and Gynecology - Großhadern, Ludwig-Maximilians-University, 81377 Munich, Germany

<sup>c</sup> Department of Obstetrics and Gynecology - Maistrasse, Ludwig-Maximilians-University, 80377 Munich, Germany

<sup>d</sup> Kinderwunsch Centrum München/Tagesklinik, Lortzingstr. 26, 81241 München, Germany

<sup>e</sup> Department of Obstetrics and Gynaecology, Medical School, University of Crete, Heraklion 71003, Greece

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## ABSTRACT

Although recurrent miscarriage (RM) affects only 1–3% of couples, it has a major influence on the wellbeing and psychosocial status of patients. Therefore, research into improved diagnosis and development of new treatment strategies is essential. In this review, we summarize current concepts on diagnosis and treatment in RM, drawing upon research reports and international guidelines to provide insights into the pathophysiology of pregnancy disrupted by repeated miscarriage. Anatomical malformations, infectious diseases, endocrine disorders, autoimmune defects as well as acquired and inherited thrombophilia are established risk factors in RM. In addition, our recent findings indicate an impact on miscarriage incidence of glycoproteins such as glycodelin, and nuclear hormone receptors such as the peroxisome proliferator-activated receptors (PPARs). Significantly reduced glycodeilin expression is associated with miscarriage, whereas up-regulation of PPARs appears to compensate for either the activated immune response or the disturbed cytotrophoblast differentiation in RM patients. There is also evidence that circulating placental microparticles are increased in a subgroup of RM patients, indicating an acquired procoagulant state even outside pregnancy. Treatment strategies like aspirin and low molecular weight heparin (LMWH) are standard medications in RM, although only a few placebo-controlled trials have proven their benefit in respect to live birth rate. There is emerging evidence that new treatment options, including drugs like TNF $\alpha$  inhibitors and granulocyte colony-stimulating factor (G-CSF) might be beneficial in some cases of RM. However, larger clinical trials must be completed to further prove or disprove benefits of these drugs in the treatment of RM patients.

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## 1. Introduction

Three or more consecutive spontaneous miscarriages with or without previous live births are defined as recurrent miscarriage (RM) and this condition affects about 1–3% of women during their reproductive years (Carrington et al., 2005). In the face of declining birth rates especially in Western Europe, there is a growing movement to offer extensive diagnostic procedures to patients suffering at least two spontaneous abortions. Primary RM refers to patients with consecutive losses and no prior successful

\* Corresponding author. Tel.: +49 6221 56 7921; fax: +49 6221 56 4009.

E-mail addresses: [bettina.toth@med.uni-heidelberg.de](mailto:bettina.toth@med.uni-heidelberg.de) (B. Toth),

[udo.jeschke@med.uni-muenchen.de](mailto:udo.jeschke@med.uni-muenchen.de) (U. Jeschke),

[nina.rogenhofer@med.uni-muenchen.de](mailto:nina.rogenhofer@med.uni-muenchen.de) (N. Rogenhofer),

[info@ivf-muenchen.de](mailto:info@ivf-muenchen.de) (W. Würfel), [makrigia@med.uoc.gr](mailto:makrigia@med.uoc.gr)

(A. Makrigiannakis).

<sup>1</sup> Tel.: +49 89 7095 6825; fax: +49 89 7095 3844.

<sup>2</sup> Tel.: +49 89 5160 4266; fax: +49 89 5160 4916.

<sup>3</sup> Tel.: +49 89 24414 40; fax: +49 89 244144 41.

<sup>4</sup> Tel.: +30 2810392131; fax: +30 2810392131.

pregnancy; secondary RM refers to losses following a live birth.

Known risk factors for RM are genetic disorders, uterine pathologies, endocrine dysfunctions, autoimmune diseases, acquired and inherited thrombophilia as well as environmental factors (Rai and Regan, 2006). Additionally, late pregnancy complications including intrauterine growth restriction (IUGR), preterm labor, and preeclampsia are associated with RM (Dolitzky et al., 2006). Still, in nearly 50% of RM patients the underlying cause remains unknown.

Normal pregnancies lead to haemostatic changes towards a procoagulatory state with an increase in concentrations of clotting factors and fibrinogen, and decreased levels of anticoagulant factors with reduced fibrinolytic activity (Brenner, 2004). It seems that a subgroup of RM patients is in a permanent “acquired” procoagulatory state as fibrin deposits are found in the intervillous space of their placentas (Rai et al., 2003). There is increasing evidence that in some RM patients, this “procoagulatory state” is linked to increased concentrations of circulating microparticles (Laude et al., 2001; Carp et al., 2004; Toth et al., 2008b; Toth, 2009).

Other possible new risk factors for RM include nuclear hormone receptors like peroxisome proliferator-activated receptors (PPAR) as well as leptin and the glycoprotein glycodelin (Toth et al., 2007, 2008c, 2009b). However, only preliminary data are available indicating a possible role of these factors in the pathophysiology of disturbed pregnancy. RM patients with acquired or inherited thrombophilia are currently treated with aspirin and/or low molecular weight heparin (LMWH), although only few placebo-controlled trials have proven their benefit concerning live birth rate in RM patients, especially in patients with antiphospholipid syndrome (APS). With regard to possible new treatment options, TNF $\alpha$  inhibitors and granulocyte colony-stimulating factor (G-CSF) seem to be two promising new drugs, although larger clinical trials have to be conducted to evaluate their potential benefits in the treatment of RM patients.

## 2. Known risk factors and treatment strategies

### 2.1. Chromosomal and single gene disorders

In 4% of couples suffering from RM, changes in the karyotypes including balanced reciprocal translocations, Robertsonian-translocations, gonosomal mosaic and inversions are found, compared to 0.2% within control couples (Franssen et al., 2005). Single gene disorders (e.g. alpha thalassemia major) can contribute to late (second and third trimester) pregnancy losses (Laurino et al., 2005). However, Stern et al. did not find any difference in incidence of abnormal karyotypes when comparing products of conception in recurrent spontaneous abortions (RSA) patients (Stern et al., 1996).

It seems that the likelihood of chromosomal disorders in RM couples is increased in women with low maternal age, a history of three or more miscarriages and affected parents or siblings (Jauniaux et al., 2006).

### 2.2. Uterine anatomic malformations

Along the most common malformations are the septate, bicornuate and didelphic uterus (Porcu et al., 2000). RM patients with septate uterus may profit from hysteroscopic metroplasty (Porcu et al., 2000; Homer et al., 2000). The pathophysiologic input of myomas on RM depends on their size and localization (Bane and Gillan, 2003). The Asherman syndrome, an acquired uterine synechia, is also associated with RM (Li et al., 2002). A significant number of preterm birth and second trimester RM are caused by cervical abnormalities due to trauma or surgical treatment (Laurino et al., 2005).

Salim et al. investigated the “true impact” of uterine malformations on RM and did not find significant differences in the relative frequency of various anomalies. However, especially with both arcuate and subseptate uteri, the length of the remaining uterine cavity was significantly shorter and the incidence of distortion was significantly higher (Salim et al., 2003).

### 2.3. Infectious diseases

Vaginal dysbiosis may lead to ascending infections, prostaglandin release, consecutive cervical insufficiency, premature rupture of the membrane and late pregnancy loss or premature birth (Li et al., 2002). Therefore, RM patients should be screened for vaginal infections and daily vaginal pH measurement should be conducted.

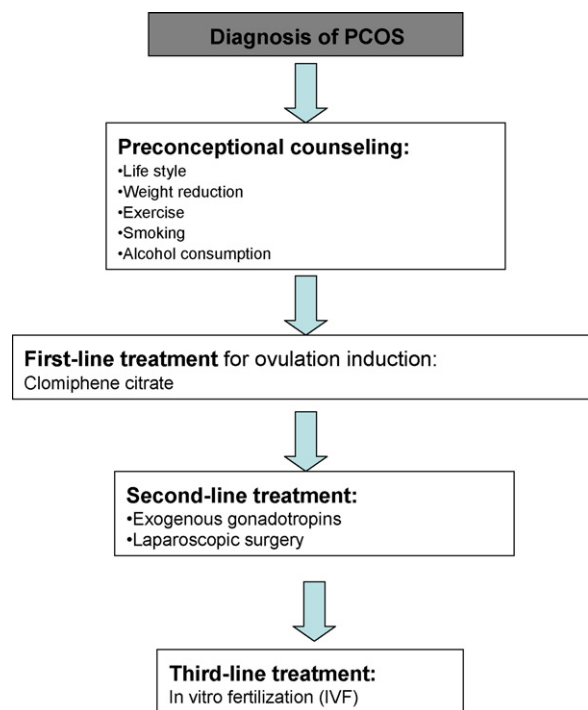
### 2.4. Psychological factors

Women with sporadic and recurrent miscarriage show high levels of pregnancy-related fear and state anxiety (Fertl et al., 2009). It seems that early pregnancy-related fear correlates significantly with complications during pregnancy and delivery (Fertl et al., 2009). Additionally, RM couples suffer from deprivation, guilt, depression and distress provoked by pregnancy loss (Sugiura-Ogasawara et al., 2002). Therefore, RM patients may profit from interventions like psychotherapy and “tender loving care” with weekly appointments and fetal scan during pregnancy (Stray-Pedersen and Stray-Pedersen, 1984; Clifford et al., 1997).

### 2.5. Endocrine disorders

Thyroid dysfunction as well as thyroid autoimmunity is linked to RM (Abalovich et al., 2002). Women during child-bearing years should be screened for TSH levels to reveal possible dysfunctions and treatment has to be started even before the onset of pregnancy. In cases of hyperprolactinemia, thyroid function should also be controlled and pituitary tumors must be excluded (Hirahara et al., 1998).

The polycystic ovary syndrome (PCOS) affects some 10% of women during their reproductive life span and it is linked to an increased risk of early RM. Proper diagnosis and treatment according to the Thessaloniki guidelines (Fig. 1) should be offered prior to conception (Jakubowicz et al., 2002; Homburg, 2006, 2008). Also, diabetes mellitus, especially Type I, is linked to RM (Christiansen et al., 2008) and



**Fig. 1.** Consensus on infertility treatment related to polycystic ovary syndrome, The Thessaloniki ESHRE/ASRM-sponsored PCOS Consensus Workshop group (2008).

levels of the glycated haemoglobin HbA1c should be within the normal range before conception.

### 2.6. Immunological risk factors

Immunological mechanisms are involved in successful implantation. Maternal adaptation of immunological responses to the implanting embryo is a key process in the establishment of the foeto-placental unit. Miscarriage may therefore be a consequence of inappropriate humoral or cellular immunological responses towards the embryo.

APS belongs to the well-known risk factors of RM and has been reported in 15% of RM patients. Antibodies against anionic phospholipids such as cardiolipins, phosphatidylserine as well as cofactors such as  $\beta$ -glycoproteins can be found disproportionately more often in RM patients as compared to healthy controls. Also functional tests for lupus anticoagulants frequently show haemostatic changes in APS patients. The diagnosis of APS requires fulfillment of the criteria defined in the international consensus statement (Miyakis et al., 2006). There is evidence that aspirin combined with LMWH significantly increases live birth rate in RM patients with APS.

#### 2.6.1. Paternal leukocyte immunization and i.v. immunoglobulins

Immunotherapy may be either active or passive. In active immunotherapy, paternal leukocyte injection is used to boost the subsequent maternal immune recognition of the developing conceptus. In passive immunotherapy, intravenous infusion of immunoglobulins

(IVIg) may neutralize circulating maternal autoantibodies, inhibit complement-mediated cytotoxicity and modulate cytokine release.

Paternal leukocyte immunization is based on the assumption that RM couples present an abnormal high HLA-conformity (HLA-sharing). Changes in CD4+CD25+ regulatory T cell numbers in peripheral blood were seen to occur after lymphocyte therapy (paternal or third party immunization) in unexplained RM patients, with higher levels of CD4+CD25(bright) T cells after therapy (Yang et al., 2009). In addition, levels of TNF $\alpha$  and IFN $\gamma$  significantly decreased after paternal immunization (Ghahresi-Fard et al., 2008). However, there are conflicting data concerning the possible benefit of paternal immunization in RM patients (Nonaka et al., 2007; Scott, 2003). Most recently, Nonaka et al. reported significantly higher successful pregnancy rates in RM patients with paternal immunization (Nonaka et al., 2007), whereas a meta-analysis showed no significant benefit of paternal leukocyte immunization on pregnancy outcome in RM patients when compared with placebo (Scott, 2003). Methodological differences (e.g. cold storage of cells or not) may partly account for the differences in study outcomes, as well as the fact that “tissue therapy” is difficult to control with respect to potency. In the future, having defined pharmaceuticals instead of “tissue therapy” is likely to help to solve these methodological problems.

Intravenous immunoglobulins are used off-label both in RM and IVF failure patients. This treatment is expensive and may have side effects such as fever, flushing, muscle pain, nausea and headache (Leong et al., 2008). It seems that a subpopulation of RM patients with  $\geq$  four miscarriages, secondary RM and >37 years might benefit from treatment with IVIg (Christiansen et al., 2002). RM patients with high numbers of CD56+CD3+ natural killer cells might also benefit from IVIg treatment (Van Den Heuvel et al., 2007). Until stronger evidence is available treatment with either paternal leukocyte immunization or IVIg should be undertaken only under controlled study conditions with a large patient cohort to achieve adequate power and appropriate stratification (Clark et al., 2001).

#### 2.6.2. Celiac disease

Celiac disease is a chronic immune-mediated gluten-dependent enteropathy induced by ingestion of gluten-containing products, characterized by intestinal malabsorption and subtotal or total atrophy of intestinal villi, which improves after exclusion of gluten from the diet. Current data, illuminating the association between celiac disease and RM are very marginal. Yet recent studies showed that there might be a correlation between the celiac disease and RM (Ludvigsson et al., 2005). It seems that RM patients with celiac disease might benefit from a gluten-free diet (Tursi et al., 2008).

### 2.7. Haemostatic changes

Hereditary thrombophilia due to mutations in the factor V and prothrombin gene, antithrombin as well as protein C and S deficiency are more prevalent in patients with disturbed pregnancy (RM, preeclampsia, late fetal loss)

than in patients with normal pregnancy (Rodger et al., 2008). Several trials emphasize the benefit of low molecular weight heparin (LMWH) in the treatment of RM patients with known hereditary thrombophilia (Ghosh et al., 2008; Brenner, 2005).

In recently released results of the heparin/aspirin (Hep-ASA) trial in Canada, surprisingly 80% of RM patients had a successful pregnancy regardless of treatment regimen (LMWH + aspirin versus aspirin alone), raising questions on the efficacy of LMWH treatment (Laskin et al., 2009).

However, inherited thrombophilia is only one of many risk factors leading to RM and is unlikely to be the unique factor that should drive treatment strategies in subsequent pregnancies. As there is evidence for benefit, coupled with a small potential for harm, LMWH could be considered as an experimental drug for RM patients until further data from controlled clinical trials are published. At present, RM patients with thrombophilia should be informed about current data concerning LMWH and pregnancy and treatment should be offered. However, clinicians should also be aware of prevention of venous thromboembolism in this subgroup of RM patients, especially during childbirth.

### 3. Possible new risk factors and treatment strategies

#### 3.1. Circulating microparticles

Circulating microparticles are increased during normal (Bretelle et al., 2003) and disturbed pregnancy conditions such as preeclampsia (Gonzalez-Quintero et al., 2004). Furthermore, there is evidence that a subgroup of RM patients is in a permanent prothrombotic state

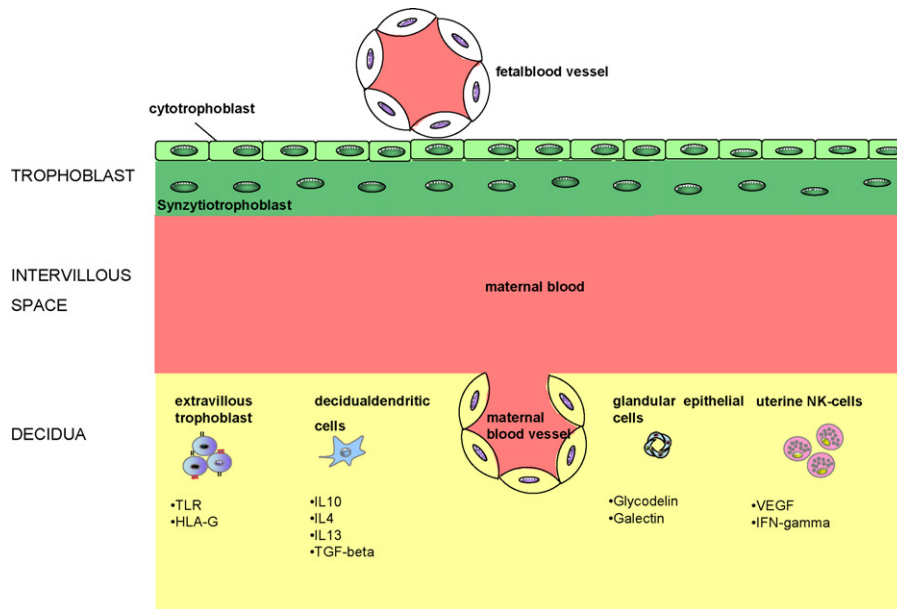
even outside pregnancy (Rai et al., 2003). Microparticles from circulating blood cells expose phosphatidylserine and thus are believed to induce coagulation (Vanwijk et al., 2003). Injection of artificial phospholipid vesicles containing phosphatidylserine into pregnant mice induced thrombosis in the placental bed and reduced birth weight, further supporting a possible role for (phosphatidylserine-positive) microparticles, especially in disturbed pregnancy (Sugimura et al., 1999).

As annexin V is found on the apical surface of syncytiotrophoblasts facing the maternal blood circulation and serves as a potent anticoagulant protein (Roberts and Schwartz, 2002), microparticles may target annexin V and reduce its anticoagulant properties (Fig. 2).

Circulating (procoagulant) microparticles in a subgroup of RM patients could be regarded as a chronic disorder, comparable to acquired thrombophilia, becoming clinically manifested during pregnancy. The extent to which microparticle concentrations are increased as a consequence of recurrent fetal losses or whether they are a major risk factor, needs to be further investigated. Whether this subgroup of RM patients might benefit from LMWH and/or aspirin during pregnancy with regard to live birth rates could also be evaluated by clinical trial.

#### 3.2. Glycoproteins

Human chorionic gonadotropin (hCG) and glycodelin are glycoproteins produced and secreted in high amounts by the trophoblast or the decidualised endometrium (Jeschke et al., 1996, 1997). Both proteins are down-regulated in RM patients (Salim et al., 2007; Toth et al., 2008c). Glycodelin carries specific and unique oligosaccharides called fucosylated LacdiNAc-structures (Dell et



**Fig. 2.** The trophoblast is exposed to maternal blood in the intervillous space. Within the decidua, a specific cellular immunologic environment is guaranteed. An extract of some cellular components being involved in the maintenance of immunotolerance at the feto-maternal interphase are presented (adapted from Moffett and Loke, 2006).

al., 1995). These glycans showed high binding affinities to the C-type lectin receptor DC-SIGN, which is probably the glycodefin receptor on dendritic cells (Scholz et al., 2008). The trophoblast-derived protein hCG carries SLeX and SLea structures (Jeschke et al., 2003b). These structures bind to E-, L- and P-selectins (Stahn et al., 2005) and hCG might prevent attachment of maternal leukocytes to the fetal syncytiotrophoblast by continuous hCG secretion during gestation (Stahn et al., 2005). In addition, both proteins are connected by a positive feedback loop. Glycodefin stimulates hCG mRNA (Reimer et al., 2000) and protein production in first trimester (Jeschke et al., 2005) and term trophoblast cells (Jeschke et al., 2003a). In addition we have shown that hCG stimulates glycodefin production in HEC1b endometrial carcinoma cells (Toth et al., 2008c). In decidual tissue of abortion patients, glycodefin expression was significantly decreased as compared to normal gestation, which was confirmed by *in situ* hybridization. Glycodefin is a major product of decidual tissue in the first trimester of human pregnancy. Reduced glycodefin expression in miscarriage could lead to increased activation of the maternal immune system, thus causing rejection of the developing fetus (Toth et al., 2008c).

### 3.3. Leptin and nuclear hormonal receptors

Leptin receptor transcripts were identified in the villos and extravillous trophoblast (Henson et al., 1998) and decreased leptin levels seem to be associated with miscarriage (Muy-Rivera et al., 2005). The homeostasis of leptin is connected to PPAR $\gamma$  by a negative feedback loop. PPAR belongs to the family of nuclear hormonal receptors which act as transcription factors with a main impact on the differentiation of adipocytes (Stumvoll and Haring, 2002; Cabrero et al., 2005). The leptin-mediated secretion of proinflammatory cytokines like IL-1 $\beta$ , IL-6, TNF $\alpha$  and prostaglandin E2 (PGE2) is inhibited through PPAR $\gamma$  activation (Lappas et al., 2005; Meirhaeghe et al., 1998). Studies on PPAR $\gamma$  null mutant mice revealed the central role of PPAR $\gamma$  in fetal development and placentation. This was further elucidated by investigations on human first trimester placenta mRNA and protein levels (Toth et al., 2007, 2008a, 2009a). PPAR $\gamma$  seems to play a major role in normal pregnancy as it facilitates the invasion of the placental trophoblast (Fournier et al., 2007; Tarrade et al., 2001).

PPAR and leptin are potential targets for new treatment strategies concerning miscarriage. The increasing knowledge about the role of PPARs and leptin in normal and disturbed pregnancy may help to improve pregnancy outcome especially in RM patients.

### 3.4. New immunological risk factors and possible treatments

Cytokines released at the feto-maternal interface play a central role in the survival of the fetus, not only by influencing the maternal immune system, but also by regulating angiogenesis and vascular development (Ashkar et al., 2000; Bulla et al., 2003). The strict model of a Th1/Th2 dysbalance as a prerequisite for RM has been challenged by the finding that T cell cytokines are released not only by T cells, but also by natural killer cells (NK cells) and dendritic cells, and growing understanding that cytokines can develop different functions in the context of the environment in which they are released (Whiteside, 1994).

Natural killer cells are amongst the major players at the feto-maternal interface. There are conflicting results with regard to activating killer cell immunoglobulin-like receptor genes (KIR) and their association with RM (Hiby et al., 2008; Moffett and Hiby, 2009). It seems that successful implantation depends on a fine balance of NK cell activation and inhibition which might be influenced by the KIR gene frequencies of the patients and their partners (Hiby et al., 2008; Faridi et al., 2009).

In an effort to identify possible new treatment options, Winger et al. enrolled 75 RM patients with elevated Th1/Th2 profile in 3 study groups (Table 1). All RM patients received LMWH. A high live birth rate was seen in the group treated with TNF $\alpha$  inhibitor, LMWH and IVIg, however only a small subgroup of RM patients was included (Winger and Reed, 2008). The same authors also studied patients with recurrent implantation failure (RIF) and were able to show significantly higher pregnancy and live birth rates in RIF patients treated with TNF $\alpha$  inhibitors (Winger et al., 2009).

Further controlled trials have to be undertaken to confirm whether or not RM patients may benefit from TNF $\alpha$  inhibitors. This is particularly critical in light of significant side effects caused by anti-TNF $\alpha$  agents including granulomatous disease, lymphoma, systemic lupus-erythematosus-like syndromes and demyelinating diseases (Pham et al., 2005).

Most recently, data have become available indicating that G-CSF may be effective in the treatment of RM of unexplained origin (Scarpellini and Sbracia, 2009). In a randomized controlled trial 68 patients with unexplained primary RM were randomized for subcutaneous G-CSF treatment (1  $\mu$ g/kg/day;  $n$  = 35) starting on day 6 after ovulation or placebo ( $n$  = 35) (Scarpellini and Sbracia, 2009). Live birth rate was 82.8% in the group treated with G-CSF, compared to 48.5% in the placebo group (Scarpellini and Sbracia, 2009). It seems that some patients with RIF may also profit from G-CSF treatment (our own unpublished data). However there are known side effects of G-CSF,

**Table 1**

TNF $\alpha$  inhibitors in the treatment of recurrent miscarriage (from Winger and Reed, 2008). The study population was divided into three groups. Group I received only LMWH, group II received IVIg and LMWH, and group III received IVIg + LMWH + TNF $\alpha$  inhibitor (Humira<sup>®</sup>).

	I, $n$ = 21 LMWH	II, $n$ = 37 IVIg + LMWH	III, $n$ = 17 LMWH + IVIg + TNF $\alpha$ inhibitor
Live birth rate	19% (4/21)	54%* (20/37)	71%** (12/17)

\* Significant differences were present concerning live birth rate between group II versus group I ( $p$  = 0.0127).

\*\* There were also significant differences with regard to live birth rate between groups III and I ( $p$  = 0.0026).

including fever, abnormal renal function, liver disease and anaphylactic or skin reactions (Meisel et al., 2009; Ernst et al., 2008).

#### 4. Summary

In developed countries, maternal age at the first pregnancy is increasing and birth rates are declining so that couples are increasingly focused on achieving one uncomplicated pregnancy. Clinicians can cope with these expectations by negotiation or by identifying any underlying problems. However, patients with miscarriages at the age of 35 years or older should seek proper diagnosis and treatment strategies even after two events. Therefore, known risk factors for RM should be excluded by intensive diagnostic workup.

To improve upon the current indiscriminate use of aspirin and LMWH for every RM patient, further clinical trials should be initiated to specify subgroups of RM patients who may benefit from anticoagulants in addition to patients with APS. Although it seems that some RM patients might benefit from IVIg treatment, the evidence has to be clarified with larger study groups. With regard to paternal leukocyte immunization, further well-designed trials are needed.

Promising new data concerning TNF $\alpha$  inhibitors and G-CSF need to be proven by larger clinical trials before we can judge if these drugs should be included in treatment recommendations.

Part of our ongoing research is to define whether or not recent findings concerning expression profiles of PPAR genes as well as data on glycodeilin expression in first trimester human pregnancy indicate possible targets for new treatment strategies. In summary, a close relationship between clinicians and researchers is required to inform both sides on developments in light of possible new risk factors and treatment strategies in RM.

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#### References

Abalovich, M., Gutierrez, S., Alcaraz, G., Maccallini, G., Garcia, A., Levalle, O., 2002. Overt and subclinical hypothyroidism complicating pregnancy. *Thyroid* 12, 63–68.

Ashkar, A.A., Di Santo, J.P., Croy, B.A., 2000. Interferon gamma contributes to initiation of uterine vascular modification, decidual integrity, and uterine natural killer cell maturation during normal murine pregnancy. *J. Exp. Med.* 192, 259–270.

Bane, A.L., Gillan, J.E., 2003. Massive perivillous fibrinoid causing recurrent placental failure. *BJOG* 110, 292–295.

Brenner, B., 2004. Haemostatic changes in pregnancy. *Thromb. Res.* 114, 409–414.

Brenner, B., 2005. Thrombophilia and pregnancy loss in first intended pregnancy. *J. Thromb. Haemost.* 3, 2176–2177.

Bretelle, F., Sabatier, F., Desprez, D., Camoin, L., Grunebaum, L., Combes, V., D'ercole, C., Dignat-George, F., 2003. Circulating microparticles: a

marker of procoagulant state in normal pregnancy and pregnancy complicated by preeclampsia or intrauterine growth restriction. *Thromb. Haemost.* 89, 486–492.

Bulla, R., Bossi, F., Radillo, O., De Seta, F., Tedesco, F., 2003. Placental trophoblast and endothelial cells as target of maternal immune response. *Autoimmunity* 36, 11–18.

Cabrero, A., Cubero, M., Llaverias, G., Alegret, M., Sanchez, R., Laguna, J.C., Vazquez-Carrera, M., 2005. Leptin down-regulates peroxisome proliferator-activated receptor gamma (PPAR-gamma) mRNA levels in primary human monocyte-derived macrophages. *Mol. Cell. Biochem.* 275, 173–179.

Carp, H., Dardik, R., Lubetsky, A., Salomon, O., Eskaraev, R., Rosenthal, E., Inbal, A., 2004. Prevalence of circulating procoagulant microparticles in women with recurrent miscarriage: a case-controlled study. *Hum. Reprod.* 19, 191–195.

Carrington, B., Sacks, G., Regan, L., 2005. Recurrent miscarriage: pathophysiology and outcome. *Curr. Opin. Obstet. Gynecol.* 17, 591–597.

Christiansen, O.B., Pedersen, B., Rosgaard, A., Husth, M., 2002. A randomized, double-blind, placebo-controlled trial of intravenous immunoglobulin in the prevention of recurrent miscarriage: evidence for a therapeutic effect in women with secondary recurrent miscarriage. *Hum. Reprod.* 17, 809–816.

Christiansen, O.B., Steffensen, R., Nielsen, H.S., Varming, K., 2008. Multifactorial etiology of recurrent miscarriage and its scientific and clinical implications. *Gynecol. Obstet. Invest.* 66, 257–267.

Clark, D.A., Coulam, C.B., Daya, S., Chaouat, G., 2001. Unexplained sporadic and recurrent miscarriage in the new millennium: a critical analysis of immune mechanisms and treatments. *Hum. Reprod. Update* 7, 501–511.

Clifford, K., Rai, R., Regan, L., 1997. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. *Hum. Reprod.* 12, 387–389.

2008. *Fertil. Steril.* 89, 505–522.

Dell, A., Morris, H.R., Easton, R.L., Panico, M., Patankar, M., Oehninger, S., Koistinen, R., Koistinen, H., Seppala, M., Clark, G.F., 1995. Structural analysis of the oligosaccharides derived from glycodeilin, a human glycoprotein with potent immunosuppressive and contraceptive activities. *J. Biol. Chem.* 270, 24116–24126.

Dolitzky, M., Inbal, A., Segal, Y., Weiss, A., Brenner, B., Carp, H., 2006. A randomized study of thromboprophylaxis in women with unexplained consecutive recurrent miscarriages. *Fertil. Steril.* 86, 362–366.

Ernst, P., Bacigalupo, A., Ringden, O., Ruutu, T., Kolb, H.J., Lawrinson, S., Skacel, T., 2008. A phase 3, randomized, placebo-controlled trial of filgrastim in patients with haematological malignancies undergoing matched-related allogeneic bone marrow transplantation. *Arch. Drug Inf.* 1, 89–96.

Faridi, R.M., Das, V., Tripathi, G., Talwar, S., Parveen, F., Agrawal, S., 2009. Influence of activating and inhibitory killer immunoglobulin-like receptors on predisposition to recurrent miscarriages. *Hum. Reprod.* 24, 1758–1764.

Fertl, K.I., Bergner, A., Beyer, R., Klapp, B.F., Rauchfuss, M., 2009. Levels and effects of different forms of anxiety during pregnancy after a prior miscarriage. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 142, 23–29.

Fournier, T., Tsatsaris, V., Handschuh, K., Evain-Brion, D., 2007. PPARs and the placenta. *Placenta* 28, 65–76.

Franssen, M.T., Korevaar, J.C., Leschot, N.J., Bossuyt, P.M., Knegt, A.C., Gerssen-Schoorl, K.B., Wouters, C.H., Hansson, K.B., Hochstenbach, R., Madan, K., Van Der Veen, F., Goddijn, M., 2005. Selective chromosome analysis in couples with two or more miscarriages: case-control study. *BMJ* 331, 137–141.

Ghaheri-Fard, B., Zolghadri, J., Kamali-Sarvestani, E., 2008. Effect of leukocyte therapy on tumor necrosis factor-alpha and interferon-gamma production in patients with recurrent spontaneous abortion. *Am. J. Reprod. Immunol.* 59, 242–250.

Ghosh, K., Shetty, S., Vora, S., Salvi, V., 2008. Successful pregnancy outcome in women with bad obstetric history and recurrent fetal loss due to thrombophilia: effect of unfractionated heparin and low-molecular weight heparin. *Clin. Appl. Thromb. Hemost.* 14, 174–179.

Gonzalez-Quintero, V.H., Smarkusky, L.P., Jimenez, J.J., Mauro, L.M., Jy, W., Hortsman, L.L., O'sullivan, M.J., Ahn, Y.S., 2004. Elevated plasma endothelial microparticles: preeclampsia versus gestational hypertension. *Am. J. Obstet. Gynecol.* 191, 1418–1424.

Henson, M.C., Swan, K.F., O'neil, J.S., 1998. Expression of placental leptin and leptin receptor transcripts in early pregnancy and at term. *Obstet. Gynecol.* 92, 1020–1028.

Hiby, S.E., Regan, L., Lo, W., Farrell, L., Carrington, M., Moffett, A., 2008. Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. *Hum. Reprod.* 23, 972–976.

- Hirahara, F., Andoh, N., Sawai, K., Hirabuki, T., Uemura, T., Minaguchi, H., 1998. Hyperprolactinemic recurrent miscarriage and results of randomized bromocriptine treatment trials. *Fertil. Steril.* 70, 246–252.
- Homburg, R., 2006. Pregnancy complications in PCOS. *Best Pract. Res. Clin. Endocrinol. Metab.* 20, 281–292.
- Homburg, R., 2008. Polycystic ovary syndrome. *Best Pract. Res. Clin. Obstet. Gynaecol.* 22 (2), 261–274, Epub 2007 Sep 5. Review.
- Homer, H.A., Li, T.C., Cooke, I.D., 2000. The septate uterus: a review of management and reproductive outcome. *Fertil. Steril.* 73, 1–14.
- Jakubowicz, D.J., Luorno, M.J., Jakubowicz, S., Roberts, K.A., Nestler, J.E., 2002. Effects of metformin on early pregnancy loss in the polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 87, 524–529.
- Jauniaux, E., Farquharson, R.G., Christiansen, O.B., Exalto, N., 2006. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. *Hum. Reprod.* 21, 2216–2222.
- Jeschke, U., Briese, V., Richter, D., Kunkel, S., 1996. Stimulation trials of trophoblast cells in vitro using PP14. *Z. Geburtshilfe Neonatol.* 200, 199–201.
- Jeschke, U., Briese, V., Richter, D., Kunkel, S., Walzel, H., Friese, K., 1997. Neue Ergebnisse zur hCG-regulation in der plazenta. *Geburtshilfe Frauenheilkd* 57, 681–684.
- Jeschke, U., Karsten, U., Reimer, T., Richter, D.U., Bergemann, C., Briese, V., Mylonas, I., Friese, K., 2005. Stimulation of hCG protein and mRNA in first trimester villous cytotrophoblast cells in vitro by glycodelin A. *J. Perinat. Med.* 33, 212–218.
- Jeschke, U., Richter, D.U., Walzel, H., Bergemann, C., Mylonas, I., Sharma, S., Keil, C., Briese, V., Friese, K., 2003a. Stimulation of hCG and inhibition of hPL in isolated human trophoblast cells in vitro by glycodelin A. *Arch. Gynecol. Obstet.* 268, 162–167.
- Jeschke, U., Stahn, R., Goletz, C., Wang, X., Briese, V., Friese, K., 2003b. hCG in trophoblast tumour cells of the cell line Jeg3 and hCG isolated from amniotic fluid and serum of pregnant women carry oligosaccharides of the sialyl Lewis X and sialyl Lewis a type. *Anticancer Res.* 23, 1087–1092.
- Lappas, M., Permezel, M., Rice, G.E., 2005. Leptin and adiponectin stimulate the release of proinflammatory cytokines and prostaglandins from human placenta and maternal adipose tissue via nuclear factor-kappaB, peroxisomal proliferator-activated receptor-gamma and extracellularly regulated kinase 1/2. *Endocrinology* 146, 3334–3342.
- Laskin, C.A., Spitzer, K.A., Clark, C.A., Crowther, M.R., Ginsberg, J.S., Hawker, G.A., Kingdom, J.C., Barrett, J., Gent, M., 2009. Low molecular Weight heparin and aspirin for recurrent pregnancy loss: results from the randomized, controlled HepASA trial. *J. Rheumatol.* 36 (2), 279–287.
- Laude, I., Rongieres-Bertrand, C., Boyer-Neumann, C., Wolf, M., Mairovitz, V., Hugel, B., Freyssinet, J.M., Frydman, R., Meyer, D., Eschwege, V., 2001. Circulating procoagulant microparticles in women with unexplained pregnancy loss: a new insight. *Thromb. Haemost.* 85, 18–21.
- Laurino, M.Y., Bennett, R.L., Saria, D.S., Baumeister, L., Doyle, D.L., Leppig, K., Pettersen, B., Resta, R., Shields, L., Uhrich, S., Varga, E.A., Raskind, W.H., 2005. Genetic evaluation and counseling of couples with recurrent miscarriage: recommendations of the National Society of Genetic Counselors. *J. Genet. Couns.* 14, 165–181.
- Leong, H., Stachnik, J., Bonk, M.E., Matuszewski, K.A., 2008. Unlabeled uses of intravenous immune globulin. *Am. J. Health Syst. Pharm.* 65, 1815–1824.
- Li, T.C., Makris, M., Tomsu, M., Tuckerman, E., Laird, S., 2002. Recurrent miscarriage: aetiology, management and prognosis. *Hum. Reprod. Update* 8, 463–481.
- Ludvigsson, J.F., Montgomery, S.M., Ekblom, A., 2005. Celiac disease and risk of adverse fetal outcome: a population-based cohort study. *Gastroenterology* 129, 454–463.
- Meirhaeghe, A., Fajas, L., Helbecque, N., Cottel, D., Lebel, P., Dallongeville, J., Deeb, S., Auwerx, J., Amouyel, P., 1998. A genetic polymorphism of the peroxisome proliferator-activated receptor gamma gene influences plasma leptin levels in obese humans. *Hum. Mol. Genet.* 7, 435–440.
- Meisel, C., Schefold, J.C., Pschowski, R., Baumann, T., Hetzger, K., Gregor, J., Weber-Carstens, S., Hasper, D., Keh, D., Zuckermann, H., Reinke, P., Volk, H.D., 2009. GM-CSF to reverse sepsis-associated immunosuppression: a double-blind randomized placebo-controlled multicenter trial. *Am. J. Respir. Crit. Care Med.* 180 (7), 585–586.
- Miyakis, S., Lockshin, M.D., Atsumi, T., Branch, D.W., Brey, R.L., Cervera, R., Derksen, R.H., Pg, D.E.G., Koike, T., Meroni, P.L., Reber, G., Shoenfeld, Y., Tincani, A., Vlachoyiannopoulos, P.G., Kritis, S.A., 2006. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J. Thromb. Haemost.* 4, 295–306.
- Moffett, A., Hiby, S., 2009. Influence of activating and inhibitory killer immunoglobulin-like receptors on predisposition to recurrent miscarriages. *Hum. Reprod.* 24, 2048–2049.
- Moffett, A., Loke, C., 2006. Immunology of placentation in eutherian mammals. *Nat. Rev. Immunol.* 6, 584–594.
- Muy-Rivera, M., Ning, Y., Frederic, I.O., Vadachkoria, S., Luthy, D.A., Williams, M.A., 2005. Leptin, soluble leptin receptor and leptin gene polymorphism in relation to preeclampsia risk. *Physiol. Res.* 54, 167–174.
- Nonaka, T., Takakuwa, K., Ooki, I., Akashi, M., Yokoo, T., Kikuchi, A., Tanaka, K., 2007. Results of immunotherapy for patients with unexplained primary recurrent abortions—prospective non-randomized cohort study. *Am. J. Reprod. Immunol.* 58, 530–536.
- Pham, T., Claudepierre, P., Deprez, X., Fautrel, B., Goupille, P., Hilliquin, P., Masson, C., Morel, J., Puechal, X., Sarau, A., Schaeverbeke, T., Mariette, X., Sibilia, J., 2005. Anti-TNF alpha therapy and safety monitoring. clinical tool guide elaborated by the Club Rhumatismes et Inflammations (CRI), section of the French Society of Rheumatology (Societe Francaise de Rhumatologie, SFR). *Joint Bone Spine* 72 (1), S1–S8.
- Porcu, G., Cravello, L., D'ercole, C., Cohen, D., Roger, V., De Montgolfier, R., Blanc, B., 2000. Hysteroscopic metroplasty for septate uterus and repetitive abortions: reproductive outcome. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 88, 81–84.
- Rai, R., Regan, L., 2006. Recurrent miscarriage. *Lancet* 368, 601–611.
- Rai, R., Tuddenham, E., Backos, M., Jivraj, S., El'gaddal, S., Choy, S., Cork, B., Regan, L., 2003. Thromboelastography, whole-blood haemostasis and recurrent miscarriage. *Hum. Reprod.* 18, 2540–2543.
- Reimer, T., Koczan, D., Briese, V., Friese, K., Richter, D., Thiesen, H.J., Jeschke, U., 2000. Absolute quantification of human chorionic gonadotropin-beta mRNA with TaqMan detection. 4. *Mol. Biotechnol.* 14, 47–57.
- Roberts, D., Schwartz, R.S., 2002. Clotting and hemorrhage in the placenta—a delicate balance. *N. Engl. J. Med.* 347, 57–59.
- Rodger, M.A., Paidas, M., McIntock, C., Middeldorp, S., Kahn, S., Martinelli, I., Hague, W., Rosene Montella, K., Greer, I., 2008. Inherited thrombophilia and pregnancy complications revisited. *Obstet. Gynecol.* 112, 320–324.
- Salim, R., Miel, J., Savvas, M., Lee, C., Jurkovic, D., 2007. A comparative study of glycodelin concentrations in uterine flushings in women with subseptate uteri, history of unexplained recurrent miscarriage and healthy controls. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 133, 76–80.
- Salim, R., Regan, L., Woelfer, B., Backos, M., Jurkovic, D., 2003. A comparative study of the morphology of congenital uterine anomalies in women with and without a history of recurrent first trimester miscarriage. *Hum. Reprod.* 18, 162–166.
- Scarpellini, F., Sbracia, M., 2009. Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: a randomized controlled trial. *Hum. Reprod.* 24 (11), 2703–2708, Epub 2009 Jul 17.
- Scholz, C., Toth, B., Brunnhuber, R., Rampf, E., Weissenbacher, T., Santoso, L., Friese, K., Jeschke, U., 2008. Glycodelin A induces a tolerogenic phenotype in monocyte-derived dendritic cells in vitro. *Am. J. Reprod. Immunol.* 60, 501–512.
- Scott, J.R., 2003. Immunotherapy for recurrent miscarriage. *Cochrane Database Syst. Rev.*, CD000112.
- Stahn, R., Goletz, S., Stahn, R., Wilmanowski, R., Wang, X., Briese, V., Friese, K., Jeschke, U., 2005. Human chorionic gonadotropin (hCG) as inhibitor of E-selectin-mediated cell adhesion. *Anticancer Res.* 25, 1811–1816.
- Stern, J.J., Dorfmann, A.D., Gutierrez-Najar, A.J., Cerrillo, M., Coulam, C.B., 1996. Frequency of abnormal karyotypes among abortuses from women with and without a history of recurrent spontaneous abortion. *Fertil. Steril.* 65, 250–253.
- Stray-Pedersen, B., Stray-Pedersen, S., 1984. Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. *Am. J. Obstet. Gynecol.* 148, 140–146.
- Stumvoll, M., Haring, H., 2002. The peroxisome proliferator-activated receptor-gamma2 Pro12Ala polymorphism. *Diabetes* 51, 2341–2347.
- Sugimura, M., Kobayashi, T., Shu, F., Kanayama, N., Terao, T., 1999. Annexin V inhibits phosphatidylserine-induced intracellular growth restriction in mice. *Placenta* 20, 555–560.
- Sugiura-Ogasawara, M., Furukawa, T.A., Nakano, Y., Hori, S., Aoki, K., Kitamura, T., 2002. Depression as a potential causal factor in subsequent miscarriage in recurrent spontaneous aborters. *Hum. Reprod.* 17, 2580–2584.
- Tarrade, A., Lai Kuen, R., Malassine, A., Tricottet, V., Blain, P., Vidaud, M., Evain-Brion, D., 2001. Characterization of human villous and extravillous trophoblasts isolated from first trimester placenta. *Lab. Invest.* 81, 1199–1211.

- Toth, B., 2009. Microparticles and female issues. *Hamostaseologie* 29, 46–50.
- Toth, B., Bastug, M., Mylonas, I., Scholz, C., Makovitzky, J., Kunze, S., Thaler, C., Friese, K., Jeschke, U., 2009a. Peroxisome proliferator-activated receptor-gamma in normal human pregnancy and miscarriage. *Acta Histochem.* 111 (4), 372–378, Epub 2009 Apr 1.
- Toth, B., Bastug, M., Mylonas, I., Scholz, C., Makovitzky, J., Kunze, S., Thaler, C., Friese, K., Jeschke, U., 2009b. Peroxisome proliferator-activated receptor-gamma in normal human pregnancy and miscarriage. *Acta Histochem.* 111, 372–378.
- Toth, B., Bastug, M., Scholz, C., Arck, P., Schulze, S., Kunze, S., Friese, K., Jeschke, U., 2008a. Leptin and peroxisome proliferator-activated receptors: impact on normal and disturbed first trimester human pregnancy. *Histol. Histopathol.* 23, 1465–1475.
- Toth, B., Hornung, D., Scholz, C., Djalali, S., Friese, K., Jeschke, U., 2007. Peroxisome proliferator-activated receptors: new players in the field of reproduction. *Am. J. Reprod. Immunol.* 58, 289–310.
- Toth, B., Nieuwland, R., Kern, M., Rogenhofer, N., Berkman, R., Rank, A., Lohse, P., Friese, K., Thaler, C.J., 2008b. Systemic changes in haemostatic balance are not associated with increased levels of circulating microparticles in women with recurrent spontaneous abortion. *Am. J. Reprod. Immunol.* 59, 159–166.
- Toth, B., Roth, K., Kunert-Keil, C., Scholz, C., Schulze, S., Mylonas, I., Friese, K., Jeschke, U., 2008c. Glycodelin protein and mRNA is downregulated in human first trimester abortion and partially upregulated in mole pregnancy. *J. Histochem. Cytochem.* 56, 477–485.
- Tursi, A., Giorgetti, G., Brandimarte, G., Elisei, W., 2008. Effect of gluten-free diet on pregnancy outcome in celiac disease patients with recurrent miscarriages. *Dig. Dis. Sci.* 53, 2925–2928.
- Van Den Heuvel, M.J., Peralta, C.G., Hatta, K., Han, V.K., Clark, D.A., 2007. Decline in number of elevated blood CD3(+) CD56(+) NKT cells in response to intravenous immunoglobulin treatment correlates with successful pregnancy. *Am. J. Reprod. Immunol.* 58, 447–459.
- Vanwijk, M.J., Vanbavel, E., Sturk, A., Nieuwland, R., 2003. Microparticles in cardiovascular diseases. *Cardiovasc. Res.* 59, 277–287.
- Whiteside, T.L., 1994. Cytokine measurements and interpretation of cytokine assays in human disease. *J. Clin. Immunol.* 14, 327–339.
- Winger, E.E., Reed, J.L., 2008. Treatment with tumor necrosis factor inhibitors and intravenous immunoglobulin improves live birth rates in women with recurrent spontaneous abortion. *Am. J. Reprod. Immunol.* 60, 8–16.
- Winger, E.E., Reed, J.L., Ashoush, S., Ahuja, S., El-Toukhy, T., Taranissi, M., 2009. Treatment with adalimumab (Humira) and intravenous immunoglobulin improves pregnancy rates in women undergoing IVF. *Am. J. Reprod. Immunol.* 61, 113–120.
- Yang, H., Qiu, L., Di, W., Zhao, A., Chen, G., Hu, K., Lin, Q., 2009. Proportional change of CD4+ CD25+ regulatory T cells after lymphocyte therapy in unexplained recurrent spontaneous abortion patients. *Fertil. Steril.* 92, 301–305.