Tumor necrosis factor inhibitors as therapeutic agents for recurrent spontaneous abortion (Review)

HONG WU[1*], QINGXIA YOU[1*], YI JIANG[1] and FANGXIANG MU[2]

[1]Department of Integrated TCM and Western Medicine, Southwest Hospital, Army Medical University, Chongqing 400038;
[2]Department of Reproductive Medicine, Lanzhou University Second Hospital, Lanzhou, Gansu 730030, P.R. China

Abstract. Recurrent spontaneous abortion (RSA) is a troublesome pregnancy disorder that manifests as sequential early pregnancy losses; its causes are diverse and complex. Among the known possible causes of RSA, the development of an immune disorder in response to the embryo appears to be the most pronounced. The imbalance between immune rejection and immune tolerance contributes to pregnancy loss in females with RSA, wherein the abnormal ratio of T helper (Th)1 cell-related cytokines [predominantly tumor necrosis factor (TNF)-α] and Th2 cell-related cytokines is a strong risk factor for RSA. TNF-α is a pro-inflammatory cytokine and TNF inhibitors have been effective in the treatment of various autoimmune diseases, such as ankylosing spondylitis, and inflammatory diseases, such as ulcerative colitis. Based on their immunomodulatory properties, TNF inhibitors have been used in the treatment of RSA to reduce the immune rejection rate and improvement in pregnancy outcomes has been observed in females suffering from RSA who were treated with TNF inhibitors. The aim of the present review was to interpret the involvement of TNF-α in the immunological disorder underlying RSA and summarize the clinical outcomes of TNF inhibitor treatment in patients with RSA.

Correspondence to: Professor Fangxiang Mu, Department of Reproductive Medicine, Lanzhou University Second Hospital, 82 Cuiyingmen, Lanzhou, Gansu 730030, P.R. China
E-mail: kyl2@163.com

*Contributed equally

Contents

1. Introduction

Recurrent spontaneous abortion (RSA) is defined as ≥3 pregnancy losses within the first 20 weeks of gestation, whereas an increasing number of researchers hold the opinion that ≥2 sequential pregnancy losses are sufficient for defining RSA. Approximately 5% of females of childbearing age suffer from RSA and the cause is unknown in over half of the cases, which makes it difficult to perform any evidence-based diagnosis and treatment (1). The etiology of RSA involves multiple factors, including immune disorders, chromosomal abnormalities, endocrine disorders and uterine abnormalities, among which immune disorders are a key factor, and efforts have been made in recent years to control RSA through agents targeting the immune system (2,3). Currently available treatments for RSA, including aspirin, anticoagulants and hormonal support, have been indicated to exert immunomodulatory effects, suggesting that targeting immunological disorders may be of value in the treatment of RSA (4).

Tumor necrosis factor (TNF)-α is a pro-inflammatory T helper (Th)1 cell cytokine, which regulates the inflammatory mechanism in various pathologies, including RSA (5-7). Regarding the immunological background of RSA, the imbalance between Th1 cytokines, particularly TNF-α, and Th2 cytokines, such as IL-10, is profound; therefore, TNF inhibitors, which are commonly used treatments for inflammatory and immune diseases, have been applied for the treatment of females with RSA (8-16). The focus of the present review was to outline the mechanistic involvement of TNF-α in RSA immune disorder and discuss clinical studies that have attempted to improve pregnancy outcomes in patients with RSA by using TNF inhibitors.

2. Immunological background in RSA

The immunological mechanism is largely altered during pregnancy in response to the development of the fetomaternal relationship. Embryos are considered as allografts to the mother, as the antigens expressed by the embryos at the fetomaternal interface are paternal and foreign, and these antigens may subsequently induce alloimmune responses in the mother (3,17); this means that the maternal immune tolerance may be broken down by the implantation of the embryo and, thus, pro-inflammatory cytokines (such as the TNF-α) may be released (Fig. 1). In addition, certain autoimmune disorders, such as the antiphospholipid syndrome (APS) and positivity for other antibodies such as anti-citrulline protein antibody, are also characterized by the overexpression of pro-inflammatory cytokines in peripheral blood mononuclear cells (PBMCs), and the overproduced TNF-α may be released into the local circulation of the implantation site, further contributing to embryonic/fetal morbidity (Fig. 1) (18,19). Approximately 5-15% of females with RSA present with APS, which refers to the presence of antiphospholipid antibodies (aPLs) and their association with venous/arterial thrombosis and hypercoagulation. The presence of aPLs has been indicated to coexist with activated CD4+ T cells and disrupted Th1/Th2 cytokine homeostasis (18). The dysregulated immune responses damage the placental villi and embryonic tissues, interrupting the implantation of the fertilized oocyte and embryonic development, resulting in pregnancy failure. For instance, the Th17 cell and regulatory T cell (Treg) imbalance (specifically, increased numbers of Th17 cells and decreased numbers of Tregs) may be an important immune factor contributing to the occurrence of RSA (20). In addition, in the case of RSA, it has been hypothesized that the adaptive immune system recognizes the alloantigen from the father, so that any further attempts at fertilization with the same partner would result in failure (3).

3. NK cells

As a critical part of the innate immune system, natural killer (NK) cells are considered as a strong risk factor for RSA and they may be classified as peripheral and uterine NK cells (1). Uterine NK cells were initially referred to as large granular lymphocytes due to the presence of granules in their cytoplasm and shared properties (CD56+) with the peripheral NK cells. However, uterine NK cells (CD16-/CD3-) are different from peripheral NK cells (CD16+/CD3+) regarding the expression of the CD16 and CD3 antigens (21). Peripheral NK cells are antiviral and antineoplastic due to their cytotoxic nature, while uterine NK cells are less cytotoxic and they produce receptors responsible for the recognition of antigens on the extravillous trophoblast surface and the secretion of cytokines (21).

Uterine NK cells are specifically reported to be associated with various reproductive disorders, including RSA, uterine fibroids and fetal growth restriction (22). Several studies using immunohistochemistry as the detection tool have reported that uterine NK cell numbers are increased in the endometrium of females with RSA at the mid-secretory phase. By contrast, studies using flow cytometry have indicated no change in uterine NK cell numbers in females with RSA (23-25). Due to the discrepancies in uterine NK cell numbers among different patients, it remains to be determined whether uterine NK cells are functional in RSA. It was previously suggested that mRNA expression is altered in endometrial uterine NK cells in early pregnancy compared with non-pregnancy NK cells (26); however, other studies indicated that endometrial uterine NK cells are non-functional in females with RSA (27,28). In addition, the adverse effects of uterine NK cells on trophoblasts, such as invasion of the trophoblast and development of trophoblast abnormalities, still require confirmation (29-31). It has been indicated that uterine NK cells are capable of promoting angiogenesis, since they also produce important pro-angiogenic factors, including vascular endothelial growth factors, placental growth factors and angiopoietin 2, and uterine NK cell deficiency caused poor spiral artery development in a mouse model (21). However, whether the involvement of uterine NK cells in angiogenesis contributes to the pathogenesis of RSA remains to be fully elucidated.

4. T cells

In immune responses, a Th1/Th2 cell ratio <10.3 is considered a safe range for successful pregnancy (32). The Th1 cells, along with the subsequent immune activation, increase the secretion of IFN-γ, IL-2 and TNF-α, which induce toxicity against the trophoblast and trigger placental injury via activating immune cells, such as NK cells. With regard to Th2 cells, they produce cytokines such as IL-4, IL-13 and IL-10, which promote trophoblast growth and favor pregnancy maintenance. The Th2 cells protect the embryo by inhibiting immune rejection by the maternal immune system (33). The evaluation of Th1/Th2 cytokines suggests that, in the first trimester of pregnancy, the concentrations of Th1 cytokines were higher in PBMCs of females with RSA compared with those with normal pregnancies (34). In addition, females who are prone to RSA but have a successful pregnancy tend to express lower Th2 cytokine levels compared with those who are not prone to RSA and suffer a pregnancy loss (35). Detailed flow cytometry data also revealed that females with RSA have significantly lower IL-10-producing CD3+/CD8+ T-cell counts but higher TNF-α-producing CD3+/CD4+ T-cell counts compared with non-pregnant fertile controls (36). The aforementioned evidence suggests the implication of Th1/Th2 imbalance in the pathogenesis of RSA (Fig. 1).

Tregs suppress the alloimmune response towards the fetus (37). In cases of allogeneic organ grafts, Tregs prevent autoimmunity, whereas in RSA, the number of available studies is limited (38). In a mouse model of spontaneous abortion, Tregs (CD4+/CD25+) from normal pregnant/non-pregnant mice inhibited lymphocyte proliferation and IFN-γ secretion *in vitro*, whereas *in vivo*, the adoptive transfer of Tregs from normal pregnant mice to maternal mice with abortion prevented fetal rejection, indicating the function of Tregs in promoting maternal tolerance of the fetus (39).

5. TNF-α in RSA

TNF-α is a Th1 pro-inflammatory cytokine that is located on chromosome 6p21.3 and acts via binding to TNF receptor (TNFR)I and TNFRII on cells. It was previously reported that TNF-α is abundant in the body fluids of patients with autoimmune
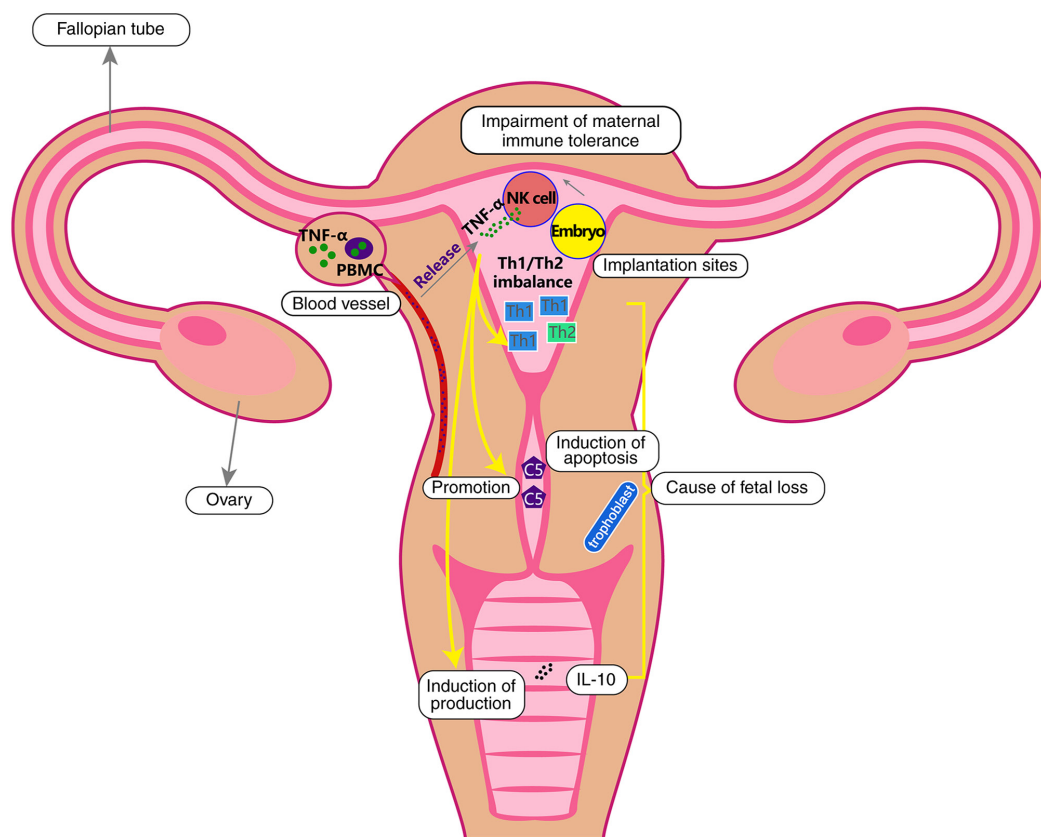
Figure 1. TNF-α origin and potential mechanism underlying its effects on RSA. After the implantation of the embryo, the maternal immune tolerance is broken down and TNF-α is then released by NK cells. In addition, in patients with autoimmune disorders, TNF-α is also released from PBMCs. TNF-α may cause RSA though disrupting the Th1/Th2 cell balance, inducing apoptosis of trophoblast cells via promoting the secretion of C5 and coagulation and promoting the production of IL-10. TNF, tumor necrosis factor; RSA, recurrent spontaneous abortion; C5, complement component 5; NK, natural killer; PBMC, peripheral blood mononuclear cell; Th, T helper.

diseases and that it is closely associated with neurodegenerative diseases (40). For instance, patients with RA or systemic lupus erythematosus frequently exhibit high levels of TNF-α and TNFRI (41). Elevated TNF-α levels were also reported in patients with multiple sclerosis (42). In addition, the levels of TNF-α along with those of other pro-inflammatory cytokines are increased during the progression of Alzheimer's disease and may be involved in neurological deterioration through nitric oxide pathways (43).

TNF-α is produced by multiple cell types, including immune cells (such as Th1 cells, NK cells, neutrophils, monocytes and macrophages) and non-immune cells (such as neuronal cells) (44). These cells are also key regulators of placentation, the dysregulation of which is a critical contributor to the pathogenesis of RSA. It has been reported that TNF-α may cause unsuccessful pregnancy through several mechanisms, including the following: i) induction of inflammation and disruption of the Th1/Th2 balance; ii) activation of the complement, causing trophoblast cell death; and iii) upregulation of IL-10 levels, leading to fetal loss (Fig. 1). In detail, TNF-α is considered to be responsible for inducing inflammation during placentation and implantation, and the balance between Th1 cytokines (predominantly TNF-α) and Th2 cytokines (predominantly IL-10) is critical for successful pregnancy (7). TNF-α may also stimulate the production of IFN-γ though promoting Treg cell differentiation to Th1 cells, while excessive expression of TNF-α and IFN-γ may activate the complement, thus leading to trophoblast cell

death (45,46). In addition, TNF-α has been reported to upregulate programmed cell death-1 levels in monocytes and induce IL-10 production, which underlies part of the immunological mechanisms involved in normal pregnancy (47), whereas in RSA, a tendency for a disequilibrium between TNF-α and IL-10 is present and the TNF-α/IL-10 ratio appears to be elevated in females with implantation failure, recurrent fetal loss and other complications, such as hypertensive syndrome (Fig. 1) (48,49). Initially, studies based on animals revealed that injecting TNF-α causes embryonic death in pregnant mice and TNF-α was indicated to be upregulated in a mouse model with a high incidence of RSA (50). In vivo, TNF-α was observed to be detrimental to mouse and cattle blastocysts due to its toxic effect, and it was also demonstrated to be involved in α-galactosylceramide (ligand expressed by Va14 NK T cells)-induced embryonic death (51).

In pregnant female patients, TNF-α is primarily produced by macrophages in the placenta during the first trimester and a high TNF-α level is a pivotal factor for adverse pregnancy conditions, including RSA, gestational hypertension and gestational diabetes mellitus (6). In addition, TNF-α gene polymorphisms may be associated with increased RSA risk. In Saudi females, the -308 G/A polymorphism in the TNF-α gene promoter was reported to be correlated with the occurrence of unexplained RSA (52). Another study reported that the -863C/A and -238G/A TNF-α polymorphisms, which are at the promoter region, are also risk factors for RSA (53).

Table I. Application of TNF inhibitors for recurrent spontaneous abortion in clinical studies.

Author (year)	Sample size, n	Treatment	Outcomes	(Refs.)
Fu et al (2019)	188	Etanercept vs. placebo	Delivery of healthy infant: Etanercept group, 89.47% vs. placebo group, 72.04%	(9)
Jerzak et al (2012)	30	Etanercept	NK cell activity was reduced after etanercept treatment	(8)
Winger et al (2009)	75	Adalimumab plus intravenous immunoglobulin vs. intravenous immunoglobulin vs. adalimumab vs. none	Live birth rate: Adalimumab plus intravenous immunoglobulin group, 73% vs. intravenous immunoglobulin group, 52% vs. adalimumab group, 50% vs. no treatment group, 0%	(16)
Winger and Reed (2008)	75	TNF inhibitor (adalimumab or etanercept) vs. anticoagulants	Live birth rate: TNF inhibitor group, 71% vs. anticoagulants group, 19%	(10)
Sills et al (2009)	1	Etanercept	Successful pregnancy and delivery after etanercept treatment	(11)
Sills et al (2001)	1	Etanercept, methotrexate and rofecoxib	Successful ovulation induction, conception and normal delivery after etanercept, methotrexate and rofecoxib treatment	(12)

TNF, tumor necrosis factor; NK, natural killer.

One study attempted to recreate the increased maternal TNF-α level by exogenously applying TNF-α to the culture media of first-trimester villous placental explants. The inflammatory antibody arrays and ELISA suggested that granulocyte/macrophage-colony-stimulating factor (CSF), C-C motif chemokine ligand 5 and IL-10 were upregulated, whereas IL-4 and macrophage CSF levels were decreased in the presence of TNF-α (6). Therefore, targeting TNF-α may be a key strategy in immunological treatments for RSA.

6. TNF-α inhibitors in RSA

TNF inhibitors suppress TNF-α binding to TNFRI and TNFRII, thus inhibiting the immune response, dendritic cells and the differentiation of Tregs, effects that are considered to be anti-abortive, and are thus applied in RSA (54-59). For instance, TNF inhibitors may suppress the expression of transcription factors, proteases or protein kinases (including NF-κB, caspases and MAPK) and inhibit the release of pro-inflammatory cytokines, chemokines and adhesion molecules (including IL-1, IL-6, IL-8, MMPs and intercellular adhesion molecule 1) through blocking TNF-α binding to TNFRI and TNFRII, and further inhibiting the activation of TNF-α that would lead to the inflammatory and immune response (54-56). On the other hand, TNF inhibitors may also restrain the activation of immune cells (including macrophages, T-cells and B-cells), inhibit the differentiation from CD4^{+} T cells to Th1 and Th17 cells and suppress the maturation of dendritic cells, which further lowers the levels of pro-inflammatory cytokines (including IL-1, IL-17 or IFN-α), thus suppressing the inflammatory reaction (57-59). In rats with abnormal maternal inflammation-induced RSA, administration of IL-10 or TNF inhibitor (etanercept) prevented pregnancy loss (13). Another study reported that treatment with etanercept blocked TNF-α activity, reduced fetal loss and restored the junctional zone ratio, placental invasion and thinning of spiral arterial walls in a syngeneic mouse model of placental insufficiency (14). It was also reported that etanercept achieved a 62% reduction of inflammation-associated coagulopathies in a rat model, which may reduce pregnancy-related disorders, including RSA (60).

In addition, since common treatments, including prednisone, heparin, aspirin, cyclosporine A or intravenous immunoglobulin (Ig), have been indicated to not be efficacious in reducing TNF-α and the cytotoxicity of NK cells in a number of patients with RSA, TNF inhibitors, which function in the innate immune system, are increasingly being investigated for their efficacy in reducing RSA. As shown in Table I, A randomized controlled trial enrolling patients with RSA with innate immune disorders reported that etanercept significantly reduced TNF-α and NK cell activity at weeks 4-10 of gestation and female patients treated with etanercept had higher live birth rates compared with those treated with placebo (9). Among females with RSA who are treated with anticoagulants, the addition of TNF inhibitors (etanercept or adalimumab) combined with intravenous immunoglobulin increased the live birth rate; however, whether the addition of TNF inhibitors alone is able to improve the live birth rate remains to be determined (10). In a study of 30 females with a history of RSA or failure of in vitro fertilization, 4 doses (25 mg) of etanercept twice weekly prior to conception significantly reduced NK cell activity, particularly among females with RSA (8). Other studies comparing adalimumab + intravenous immunoglobulin with intravenous immunoglobulin- or adalimumab-alone (16) and evaluating the efficacy of etanercept-alone or in combination with methotrexate and rofecoxib in patients with RSA are shown in Table I (8-12,16).

For the subsequent analysis of the currently available data on the application of TNF inhibitors for RSA, the use of TNF inhibitors, the treatment regimens, complications and clinical follow-up were compared among the available

clinical studies on RSA (8-12,16) and various noteworthy points became apparent: i) Among all of the TNF inhibitors, etanercept was the most frequently used in clinical practice for the treatment of RSA. In detail, 5 out of the 6 studies used etanercept for the treatment of RSA, while only two studies used adalimumab (adalimumab and etanercept were both used in one study) (10)); in addition, the use of etanercept achieved a higher rate of healthy deliveries compared with adalimumab; ii) TNF inhibitor monotherapy was more commonly used than combined therapy, although the results suggested that combined therapy may achieve a higher live birth rate; iii) in terms of complications, only a small number of studies performed to date have evaluated the complications among patients with RSA who received TNF inhibitor treatment and, thus, no relevant data are provided in the present review; and iv) regarding the clinical follow-up, most studies terminated the follow-up at live birth or the occurrence of abortion, with the exception of one study that continued the follow-up until 3 months after the delivery. The most attractive endpoint is the live birth rate, which ranged from 50 to 89.47% in patients with RSA after treatment with TNF inhibitors.

The safety of TNF inhibitors during pregnancy has long been studied, since they are widely applied drugs for rheumatoid arthritis (RA), including patients with RA during pregnancy. Successful ovulation induction, conception and normal delivery are reported among females receiving long-term TNF inhibitors as anti-cytokine treatment for RA (11,12). Regarding pregnant females with inflammatory bowel disease, infliximab and other TNF inhibitors have been reported to be safe, as there was no reported increased incidence of spontaneous abortion or other adverse pregnancy outcomes (61). However, according to the official guidelines of the German Society of Gynecology and Obstetrics, the Austrian Society of Gynecology and Obstetrics and the Swiss Society of Gynecology and Obstetrics, the use of TNF inhibitors should be reserved for specific conditions, such as Crohn's disease and other autoimmune diseases, due to possible side effects such as skin irritation and drug-induced lupus (15).

7. Progress of TNF inhibitor application

As one of the initially approved TNF inhibitors, etanercept, a fusion protein comprised of the extracellular domain of human TNFRII receptor and the Fc region of human IgG1, was approved for RA treatment in the US and Europe in 1998 (62,63). In the same year, infliximab, a chimeric monoclonal antibody that contains a murine antigen-binding region joined to the human IgG1 constant region, was approved for the treatment of Crohn's disease in the US and soon after (in 1999) in Europe, launching the new era of TNF inhibitors in bowel diseases (64). Both etanercept and infliximab were later applied and approved for other inflammatory and autoimmune diseases, such as ankylosing spondylitis and ulcerative colitis. Etanercept is administered as a weekly subcutaneous injection, while infliximab is delivered intravenously in-hospital (63,64). Following the success of the aforementioned treatments, the fully human antibodies adalimumab and golimumab were produced by immunizing genetically engineered mice with human TNF-α, which solved the problem of the immunogenic nature of infliximab (a chimeric monoclonal antibody), as well as its relatively severe adverse effects

such as the production of anti-antibody or the occurrence of allergy (62,63,65-71). The release of TNF inhibitors to the market has revolutionized the treatment of RA, ankylosing spondylitis, psoriasis, Chron's disease and ulcerative colitis, particularly RA and ankylosing spondylitis, for which TNF inhibitors have become a standard treatment, and they are generally well-tolerated, with long-term efficacy (72). In addition, the open-label extensions of clinical trials of TNF inhibitors and observations post-market release also support their efficacy and safety.

TNF inhibitors are among the best-selling drugs worldwide (73). However, the cost of these biologicals is high, with an annual cost per patient of >20,000 USD. Therefore, in addition to the TNF inhibitors already launched, several other biologicals are available, including biosimilars and novel drugs under investigation. For instance, a humanized anti-TNF monoclonal antibody, SSS-07, is currently under investigation in a clinical trial for RA in China [National Clinical Trial (NCT) no. NCT02460393] (74). In addition, the approved biosimilars of infliximab include CT-P13 (66) and SB2 (67); the approved biosimilars of etanercept include GP2015 (68) and SB4 (62); and the approved biosimilars of adalimumab include ABP501, BI695501, GP2017 and SB5 (69). So far, >20 newly developed anti-TNF biologicals were in the pipeline, as well as new indications of the already approved drugs (73).

With research efforts focused on drug development, the dosing of TNF inhibitors may become more practicable and cost-effective in clinical practice and their application may be expanded to immunomodulatory conditions other than the commonly approved indications, e.g., the immune disorder underlying RSA.

8. Concluding remarks

There are currently numerous studies on the application of TNF inhibitors for the treatment of autoimmune and inflammatory diseases. Considering the contribution of immunological disorders to the mechanism underlying RSA, the application of TNF inhibitors in patients with RSA aims to target the immune aspect of this condition. In pre-clinical studies, TNF inhibitors have been indicated to successfully reduce NK cell numbers and Th1-related cytokine levels, which are risk factors for RSA, and clinical attempts have already achieved an improvement in the live birth rate. However, therapies for RSA using TNF inhibitors still lack sufficient supportive data. In addition, the development of TNF inhibitors in the pharmaceutical industry is still growing, which may be promising for the future clinical application of TNF inhibitors in RSA.

Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

FM conceived and supervised the study. HW and QY participated in the acquisition of data and collated the data. YJ analyzed the data and wrote, edited and revised the manuscript. All authors participated in revising the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Toth B, Vomstein K, Togawa R, Böttcher B, Hudalla H, Strowitzki T, Daniel V and Kuon RJ: The impact of previous live births on peripheral and uterine natural killer cells in patients with recurrent miscarriage. Reprod Biol Endocrinol 17: 72, 2019.
2. Ozkan ZS, Deveci D, Simsek M, Ilhan F, Risvanli A and Sapmaz E: What is the impact of SOCS3, IL-35 and IL17 in immune pathogenesis of recurrent pregnancy loss? J Matern Fetal Neonatal Med 28: 324-328, 2015.
3. Pei CZ, Kim YJ and Baek KH: Pathogenetic factors involved in recurrent pregnancy loss from multiple aspects. Obstet Gynecol Sci 62: 212-223, 2019.
4. Ou H and Yu Q: Efficacy of aspirin, prednisone, and multivitamin triple therapy in treating unexplained recurrent spontaneous abortion: A cohort study. Int J Gynaecol Obstet 148: 21-26, 2020.
5. Alijotas-Reig J, Esteve-Valverde E, Ferrer-Oliveras R, Llurba E and Gris JM: Tumor Necrosis Factor-Alpha and pregnancy: Focus on biologics. An updated and comprehensive review. Clin Rev Allergy Immunol 53: 40-53, 2017.
6. Siwetz M, Blaschitz A, El-Heliebi A, Hiden U, Desoye G, Huppertz B and Gauster M: TNF-alpha alters the inflammatory secretion profile of human first trimester placenta. Lab Invest 96: 428-438, 2016.
7. Saito S, Nakashima A, Shima T and Ito M: Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol 63: 601-610, 2010.
8. Jerzak M, Ohams M, Gorski A and Baranowski W: Etanercept immunotherapy in women with a history of recurrent reproductive failure. Ginekol Pol 83: 260-264, 2012.
9. Fu J, Li L, Qi L and Zhao L: A randomized controlled trial of etanercept in the treatment of refractory recurrent spontaneous abortion with innate immune disorders. Taiwan J Obstet Gynecol 58: 621-625, 2019.
10. Winger EE and Reed JL: 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, 2008.
11. Sills ES, Walsh DJ, Shkrobot LV, Palermo GD and Walsh AP: Clinical experience with intravenous immunoglobulin and tnf-a inhibitor therapies for recurrent pregnancy loss. Ulster Med J 78: 57-58, 2009.
12. Sills ES, Perloe M, Tucker MJ, Kaplan CR and Palermo GD: Successful ovulation induction, conception, and normal delivery after chronic therapy with etanercept: A recombinant fusion anti-cytokine treatment for rheumatoid arthritis. Am J Reprod Immunol 46: 366-368, 2001.
13. Renaud SJ, Cotechini T, Quirt JS, Macdonald-Goodfellow SK, Othman M and Graham CH: Spontaneous pregnancy loss mediated by abnormal maternal inflammation in rats is linked to deficient uteroplacental perfusion. J Immunol 186: 1799-1808, 2011.
14. Gelber SE, Brent E, Redecha P, Perino G, Tomlinson S, Davisson RL and Salmon JE: Prevention of defective placentation and pregnancy loss by blocking innate immune pathways in a syngeneic model of placental insufficiency. J Immunol 195: 1129-1138, 2015.
15. Toth B, Wurfel W, Bohlmann M, Zschocke J, Rudnik-Schöneborn S, Nawroth F, Schleußner E, Rogenhofer N, Wischmann T, von Wolff M, *et al*: Recurrent Miscarriage: Diagnostic and Therapeutic Procedures. Guideline of the DGGG, OEGGG and SGGG (S2k-Level, AWMF Registry Number 015/050). Geburtshilfe Frauenheilkd 78: 364-381, 2018.
16. Winger EE, Reed JL, Ashoush S, Ahuja S, El-Toukhy T and Taranissi M: Treatment with adalimumab (Humira) and intravenous immunoglobulin improves pregnancy rates in women undergoing IVF. Am J Reprod Immunol 61: 113-120, 2009.
17. Baines MG, Duclos AJ, Antecka E and Haddad EK: Decidual infiltration and activation of macrophages leads to early embryo loss. Am J Reprod Immunol 37: 471-477, 1997.
18. Garrido-Gimenez C and Alijotas-Reig J: Recurrent miscarriage: Causes, evaluation and management. Postgrad Med J 91: 151-162, 2015.
19. Boomsma CM, Kavelaars A, Eijkemans MJ, Lentjes EG, Fauser BC, Heijnen CJ and Macklon NS: Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. Hum Reprod 24: 1427-1435, 2009.
20. Guo Z, Xu Y, Zheng Q, Liu Y and Liu X: Analysis of chromosomes and the T helper 17 and regulatory T cell balance in patients with recurrent spontaneous abortion. Exp Ther Med 19: 3159-3166, 2020.
21. Quenby S and Farquharson R: Uterine natural killer cells, implantation failure and recurrent miscarriage. Reprod Biomed Online 13: 24-28, 2006.
22. Lash GE and Bulmer JN: Do uterine natural killer (uNK) cells contribute to female reproductive disorders? J Reprod Immunol 88: 156-164, 2011.
23. Tuckerman E, Laird SM, Prakash A and Li TC: Prognostic value of the measurement of uterine natural killer cells in the endometrium of women with recurrent miscarriage. Hum Reprod 22: 2208-2213, 2007.
24. Tuckerman E, Mariee N, Prakash A, Li TC and Laird S: Uterine natural killer cells in peri-implantation endometrium from women with repeated implantation failure after IVF. J Reprod Immunol 87: 60-66, 2010.
25. Shimada S, Kato EH, Morikawa M, Iwabuchi K, Nishida R, Kishi R, Onoé K, Minakami H and Yamada H: No difference in natural killer or natural killer T-cell population, but aberrant T-helper cell population in the endometrium of women with repeated miscarriage. Hum Reprod 19: 1018-1024, 2004.
26. Manaster I and Mandelboim O: The unique properties of human NK cells in the uterine mucosa. Placenta 29 (Suppl A): S60-S66, 2008.
27. Manaster I, Mizrahi S, Goldman-Wohl D, Sela HY, Stern-Ginossar N, Lankry D, Gruda R, Hurwitz A, Bdolah Y, Haimov-Kochman R, *et al*: Endometrial NK cells are special immature cells that await pregnancy. J Immunol 181: 1869-1876, 2008.
28. Kopcow HD, Allan DS, Chen X, Rybalov B, Andzelm MM, Ge B and Strominger JL: Human decidual NK cells form immature activating synapses and are not cytotoxic. Proc Natl Acad Sci USA 102: 15563-15568, 2005.
29. Guimond MJ, Wang B and Croy BA: Engraftment of bone marrow from severe combined immunodeficient (SCID) mice reverses the reproductive deficits in natural killer cell-deficient tg epsilon 26 mice. J Exp Med 187: 217-223, 1998.
30. Rai R, Sacks G and Trew G: Natural killer cells and reproductive failure-theory, practice and prejudice. Hum Reprod 20: 1123-1126, 2005.
31. Quenby S, Vince G, Farquharson R and Aplin J: Recurrent miscarriage: A defect in nature's quality control? Hum Reprod 17: 1959-1963, 2002.
32. Nakagawa K, Kwak-Kim J, Ota K, Kuroda K, Hisano M, Sugiyama R and Yamaguchi K: Immunosuppression with tacrolimus improved reproductive outcome of women with repeated implantation failure and elevated peripheral blood TH1/TH2 cell ratios. Am J Reprod Immunol 73: 353-361, 2015.
33. Raghupathy R, Makhseed M, Azizieh F, Hassan N, Al-Azemi M and Al-Shamali E: Maternal Th1- and Th2-type reactivity to placental antigens in normal human pregnancy and unexplained recurrent spontaneous abortions. Cell Immunol 196: 122-130, 1999.

34. Darmochwal-Kolarz D, Rolinski J, Leszczynska-Goarzelak B and Oleszczuk J: The expressions of intracellular cytokines in the lymphocytes of preeclamptic patients. Am J Reprod Immunol 48: 381-386, 2002.
35. Kwak-Kim JYH, Gilman-Sachs A and Kim CE: T helper 1 and 2 immune responses in relationship to pregnancy, nonpregnancy, recurrent spontaneous abortions and infertility of repeated implantation failures. Chem Immunol Allergy 88: 64-79, 2005.
36. Kwak-Kim JY, Chung-Bang HS, Ng SC, Ntrivalas EI, Mangubat CP, Beaman KD, Beer AE and Gilman-Sachs A: Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. Hum Reprod 18: 767-773, 2003.
37. Wang WJ, Hao CF, Qu QL, Wang X, Qiu LH and Lin QD: The deregulation of regulatory T cells on interleukin-17-producing T helper cells in patients with unexplained early recurrent miscarriage. Hum Reprod 25: 2591-2596, 2010.
38. Zelenika D, Adams E, Humm S, Lin CY, Waldmann H and Cobbold SP: The role of CD4+ T-cell subsets in determining transplantation rejection or tolerance. Immunol Rev 182: 164-179, 2001.
39. Zenclussen AC, Gerlof K, Zenclussen ML, Sollwedel A, Bertoja AZ, Ritter T, Kotsch K, Leber J and Volk HD: Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: Adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. Am J Pathol 166: 811-822, 2005.
40. Yan L, Zheng D and Xu RH: Critical role of tumor necrosis factor signaling in mesenchymal stem cell-based therapy for autoimmune and inflammatory diseases. Front Immunol 9: 1658, 2018.
41. Gabay C, Cakir N, Moral F, Roux-Lombard P, Meyer O, Dayer JM, Vischer T, Yazici H and Guerne PA: Circulating levels of tumor necrosis factor soluble receptors in systemic lupus erythematosus are significantly higher than in other rheumatic diseases and correlate with disease activity. J Rheumatol 24: 303-308, 1997.
42. Sharief MK and Hentges R: Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. N Engl J Med 325: 467-472, 1991.
43. Belkhelfa M, Rafa H, Medjeber O, Arroul-Lammali A, Behairi N, Abada-Bendib M, Makrelouf M, Belarbi S, Masmoudi AN, Tazir M and Touil-Boukoffa C: IFN-γ and TNF-α are involved during Alzheimer disease progression and correlate with nitric oxide production: A study in Algerian patients. J Interferon Cytokine Res 34: 839-847, 2014.
44. Idriss HT and Naismith JH: TNF alpha and the TNF receptor superfamily: Structure-function relationship(s). Microsc Res Tech 50: 184-195, 2000.
45. Clark DA: Immunological factors in pregnancy wastage: Fact or fiction. Am J Reprod Immunol 59: 277-300, 2008.
46. Clark DA: Anti-TNFalpha therapy in immune-mediated subfertility: State of the art. J Reprod Immunol 85: 15-24, 2010.
47. Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, Hill BJ, Noto A, Ancuta P, Peretz Y, et al: Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. Nat Med 16: 452-459, 2010.
48. Wedekind L and Belkacemi L: Altered cytokine network in gestational diabetes mellitus affects maternal insulin and placental-fetal development. J Diabetes Complications 30: 1393-1400, 2016.
49. Banerjee P, Jana SK, Pasricha P, Ghosh S, Chakravarty B and Chaudhury K: Proinflammatory cytokines induced altered expression of cyclooxygenase-2 gene results in unreceptive endometrium in women with idiopathic recurrent spontaneous miscarriage. Fertil Steril 99: 179-187 e2, 2013.
50. Tangri S and Raghupathy R: Expression of cytokines in placentas of mice undergoing immunologically mediated spontaneous fetal resorptions. Biol Reprod 49: 850-856, 1993.
51. Ito K, Karasawa M, Kawano T, Akasaka T, Koseki H, Akutsu Y, Kondo E, Sekiya S, Sekikawa K, Harada M, et al: Involvement of decidual Valpha14 NKT cells in abortion. Proc Natl Acad Sci USA 97: 740-744, 2000.
52. Alkhuriji AF, Alhimaidi AR, Babay ZA and Wary AS: The relationship between cytokine gene polymorphism and unexplained recurrent spontaneous abortion in Saudi females. Saudi Med J 34: 484-489, 2013.
53. Aboutorabi R, Behzadi E, Sadegh MJ, Fatehi SP, Semsarzadeh S, Zarrin Y, Kazemi M, Rafiee L and Mostafavi FS: The study of association between polymorphism of TNF-α gene's promoter region and recurrent pregnancy loss. J Reprod Infertil 19: 211-218, 2018.
54. Gerriets V, Bansal P, Goyal A and Khaddour K: Tumor Necrosis Factor Inhibitors. In: StatPearls [Internet]. StatPearls Publishing, Treasure Island, FL, 2021
55. Wang T and He C: TNF-α and IL-6: The link between immune and bone system. Curr Drug Targets 21: 213-227, 2020.
56. Zhang Y, Liu H, Tang W, Qiu Q and Peng J: Resveratrol prevents TNF-α-induced VCAM-1 and ICAM-1 upregulation in endothelial progenitor cells via reduction of NF-κB activation. J Int Med Res 48: 300060520945131, 2020.
57. Brunner C, Seiderer J, Schlamp A, Bidlingmaier M, Eigler A, Haimerl W, Lehr HA, Krieg AM, Hartmann G and Endres S: Enhanced dendritic cell maturation by TNF-alpha or cytidine-phosphate-guanosine DNA drives T cell activation in vitro and therapeutic anti-tumor immune responses in vivo. J Immunol 165: 6278-6286, 2000.
58. Psarras A, Antanaviciute A, Alase A, Carr I, Wittmann M, Emery P, Tsokos GC and Vital EM: TNF-α regulates human plasmacytoid dendritic cells by suppressing IFN-α production and enhancing T cell activation. J Immunol 206: 785-796, 2021.
59. Conrad C, Di Domizio J, Mylonas A, Belkhodja C, Demaria O, Navarini AA, Lapointe AK, French LE, Vernez M and Gilliet M: TNF blockade induces a dysregulated type I interferon response without autoimmunity in paradoxical psoriasis. Nat Commun 9: 25, 2018.
60. Falcon BJ, Cotechini T, Macdonald-Goodfellow SK, Othman M and Graham CH: Abnormal inflammation leads to maternal coagulopathies associated with placental haemostatic alterations in a rat model of foetal loss. Thromb Haemost 107: 438-447, 2012.
61. Dignass AU, Hartmann F, Sturm A and Stein J: Management of inflammatory bowel diseases during pregnancy. Dig Dis 27: 341-346, 2009.
62. Pelechas E and Drosos AA: Etanercept biosimilar SB-4. Expert Opin Biol Ther 19: 173-179, 2019.
63. Spencer-Green G: Etanercept (Enbrel): Update on therapeutic use. Ann Rheum Dis 59 (Suppl 1): i46-i49, 2000.
64. Elliot MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Katasikis P, Brennan FM, Bijl H, Ghrayeb J and Woody JN: Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. Arthritis Rheum 58 (2 Suppl): S92-S101, 2008.
65. Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, et al: Maintenance infliximab for Crohn's disease: The ACCENT I randomised trial. Lancet 359: 1541-1549, 2002.
66. Yoo DH, Prodanovic N, Jaworski J, Miranda P, Ramiterre E, Lanzon A, Baranauskaite A, Wiland P, Abud-Mendoza C, Oparanov B, et al: Efficacy and safety of CT-P13 (biosimilar infliximab) in patients with rheumatoid arthritis: Comparison between switching from reference infliximab to CT-P13 and continuing CT-P13 in the PLANETRA extension study. Ann Rheum Dis 76: 355-363, 2017.
67. Zheng MK, Shih DQ and Chen GC: Insights on the use of biosimilars in the treatment of inflammatory bowel disease. World J Gastroenterol 23: 1932-1943, 2017.
68. Deeks ED: GP2015: An etanercept biosimilar. BioDrugs 31: 555-558, 2017.
69. Fiorino G, Gilardi D, Correale C, Furfaro F, Roda G, Loy L, Argollo M, Allocca M, Peyrin-Biroulet L and Danese S: Biosimilars of adalimumab: The upcoming challenge in IBD. Expert Opin Biol Ther 19: 1023-1030, 2019.
70. Nelson AL, Dhimolea E and Reichert JM: Development trends for human monoclonal antibody therapeutics. Nat Rev Drug Discov 9: 767-774, 2010.
71. Deeks ED: Certolizumab pegol: A review in inflammatory autoimmune diseases. BioDrugs 30: 607-617, 2016.
72. Maxwell LJ, Zochling J, Boonen A, Singh JA, Veras MM, Tanjong Ghogomu E, Benkhalti Jandu M, Tugwell P and Wells GA: TNF-alpha inhibitors for ankylosing spondylitis. Cochrane Database Syst Rev CD005468, 2015.
73. Steeland S, Libert C and Vandenbroucke RE: A new venue of TNF targeting. Int J Mol Sci 19: 1442, 2018.
74. U.S. National Library of Medicine (NIH): A Trial With Humanized TNFα Monoclonal Antibody Injection by Single Dose and Dose Escalation in Healthy Subjects. ClinicalTrials.gov Identifier: NCT02460393. NIH, Bethesda, MD, 2015. https://clinicaltrials.gov/ct2/show/NCT02460393. Accessed June 2, 2015.