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# Targeting CD3-CD16+CD56+ NK Cells and NK Cell Activity by Intralipid in the Management of Reproductive Failure

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**Abstract:** Immunological risk factors in recurrent pregnancy loss include autoantibodies, alterations in NK cell number and function, regulatory T cells, the human leukocyte antigen system (HLA), etc., where the treatment options aim to regulate immune dysfunction. Intralipid is a synthetic product traditionally used as a dietary supplement consisting of soybean oil combined with refined egg phospholipids. It has been shown that intralipid exerts physiologic activities, including altering immunological functions, that may benefit patients with certain types of infertility. In this review, we summarize the current state of the art of targeting NK cells and NK cell activity in women with implantation failure or/and recurrent pregnancy loss. We focus on intralipid mechanisms of action and outcomes of clinical trials regarding the efficacy and safety of intralipid infusions in women with reproductive failure. More studies are needed to reveal all the aspects of the safety and effectiveness of intralipid administration in reproductive failure treatment.

**Keywords:** intralipid; infertility; reproductive failure; recurrent pregnancy loss; recurrent fetal loss; implantation failure; IVF; NK cells; NKT cells; immune cells

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**Citation:** Velikova, T.; Tomov, L.; Nikolaev, G. Targeting CD3-CD16+CD56+ NK Cells and NK Cell Activity by Intralipid in the Management of Reproductive Failure. *Targets* **2024**, *2*, 295–306. https://doi.org/10.3390/targets2040017

Academic Editor: Fátima Martel

Received: 3 June 2024
Revised: 1 October 2024
Accepted: 4 October 2024
Published: 8 October 2024



## 1. Introduction

Feto-maternal crosstalk during implantation remains elusive, although recent advancements in molecular biology have shed insight onto some of the factors involved in a successful pregnancy [1]. For example, the unusual expression of MHC, certain hormones and cytokines, and the distributions and functions of uterine natural killer (uNK) cells are all critical features of feto-maternal immunotolerance during implantation and pregnancy [1].

Immunological risk factors for reproductive failure are entering the scientific spotlight, with roughly 50% of idiopathic cases of unsuccessful pregnancies. However, worldwide recommendations and international guidelines from the American Society of Reproductive Medicine (ASRM), the Royal College of Obstetricians and Gynecologists (RCOG), the European Society of Reproduction and Embryology (ESHRE), and the German/Austrian/Swiss Society of Obstetrics and Gynecology (DGGG/OEGGG/SGGG), seldom focus on the immunological aspects of infertility [2]. The current state of immunological risk factors in recurrent pregnancy loss (RPL) is focused on immunological phenomena such as autoantibodies, NK cells, NKT cells, regulatory T cells, plasma cells, dendritic cells, and the human leukocyte antigen system (HLA). In line with this, a few treatment options have been discussed, such as corticosteroids, intralipid, intravenous immunoglobulins (IVIGs), aspirin, low molecular heparin, etc. [2].

However, miscarriage causes are usually complicated and can be challenging to identify. Chromosomal abnormalities mainly cause early pregnancy failure, and the likelihood of a euploid embryo diminishes with the age of the mother. Thrombophilic abnormalities,

endocrine disorders, infections, and anatomical issues could also play a role. Although implantation failure is a distinct reproductive issue, some therapies address it [3]. Furthermore, the embryo or endometrium quality is the primary cause of recurrent implantation failure (RIF), while endometrial receptivity and implantation window abnormalities may also present. It is still debatable whether immunological variables contribute to unsuccessful implantation and miscarriages [4].

In this review, we summarize the current state of the art regarding immunological risk factors for RPL, with particular attention to the mechanisms of intralipid action on CD3-CD16+CD56+ NK cells and NK cell activity. We also present the recent outcomes of clinical trials regarding the efficacy and safety of intralipid infusions in women with reproductive failure and increased NK cells. Since the advantages and effectiveness of intravenous intralipid treatment for patients with a poor reproductive history are up for debate, the topic is somewhat controversial. It is often reported that intralipid use is not supported by reliable evidence. However, emerging studies suggest potential benefits in specific patient populations, highlighting the need for more rigorous, large-scale clinical trials. Understanding the nuances of these findings is crucial for developing more effective and personalized treatments for patients with reproductive failure. Therefore, we summarize the existing data in the literature for targeting increased NK cells and NK cell activity in women with RPL, implantation failure, and other reproductive challenges by intralipid infusions.

## 2. Immune Cells and Infertility

RIF is thought to reflect the failure of the uterine endometrial lining to attain a sufficiently receptive state. It is caused by a failure of the uterine immune system to maintain immune tolerance [5,6]. If the thrombophilia tests are normal, patients with reproductive failure should be evaluated for immunological causes of infertility.

### *2.1. NK Cells and Infertility*

According to recent studies [7,8], elevated numbers of $CD56^{dim}$ cells and NK cytotoxicity in peripheral blood may be critical contributors to both RPL and in vitro fertilization (IVF) failure. The predictive value of preconceptional peripheral blood NK cell activity has recently been evaluated. It has been reported that measuring peripheral blood NK cells does not help to evaluate RPL risk directly. However, various reports have documented the usefulness of measuring pre-pregnancy NK cells to indicate reproductive success. CD56 and CD16 NK cells can be subdivided into two primary subsets: $CD56^{bright}CD16^{dim}$ (less toxic, producing cytokines, often found in secondary lymphoid tissues) and $CD56^{dim}CD16^{bright}$ (highly cytotoxic, predominant in the peripheral blood) [9]. During the luteal phase and early pregnancy, $CD56^{bright}CD16^{dim}$ cells are abundant in the endometrium, maintaining vascular remodeling, promoting tissue repair, and modulating immune response. However, NK cells interact with trophoblast cells, ensuring normal pregnancy [10–12]; therefore, NK cell numbers and cytotoxicity should be managed carefully for therapeutic purposes.

Studies have shown that women with RPL and RIF have altered numbers and functionality of specific immune cells (such as uterine (u)NK cells) in their endometrium [13]. Even while NK cell counts are known to rise during the first trimester of pregnancy, RPL and RIF appear to be linked to either an excessive rise or fall in endometrial NK cell counts [13]. Furthermore, RIF and RPL are associated with decreased regulatory NK cells and increased cytotoxic NK cells when examining various NK cell subtypes. Knowing these immune dysregulations may help us to identify particular therapeutic targets and offer insight into possible diagnostic indicators.

### *2.2. Targeting NK Cells in Reproductive Failure*

Targeting NK cells in reproductive failure is a routine practice, although the medication protocols are not uniform [14]. The studies in this area indicate that there may be a drop in the uNK counts after prednisolone treatment. However, this drop does not allow all

patients to fall into a normal uNK reference range. Furthermore, no improvement in the pregnancy outcome has been seen despite the somewhat restricted and non-extensive mitigation of elevated uNK levels in the uterine environment. Contradictory evidence seems to support or refute the use of prednisolone in reducing the harmful amounts of uNK cells in both the RIF and miscarriage rate (MR).

It has been suggested in the literature that intralipid treatment can be used to lessen the adverse effects of increased uNK cell count and activity, as well as IVIGs [15]. It is assumed that IVIGs both stimulate changes in cytokine production and attenuate the activity of NK cells. Because IVIGs inhibit the cytotoxic activity of many immune cells, including T and B lymphocytes, dendritic cells, NK cells, etc., both in vitro and in vivo, they are therefore employed as "immunomodulatory" drugs in various immunological and inflammatory illnesses, and also in reproductive medicine [16,17].

### *2.3. NKT Cells and Infertility*

However, there are also numerous immune cell populations in the decidua, except uNK cells, dendritic cells, T cells, etc. [18], modulated by a complex array of cytokines and chemokines in the endometrium. NKT cells are also discussed as being involved in recurrent fetal loss or implantation failure when increased in peripheral blood and the uterus [19]. NKT cells are a specific subset of T cells that possess characteristics of both innate and adaptive immune cells. Uterine NKT cells support tissue homeostasis and control regional immune responses in the endometrium. They interact with other immune cells and produce cytokines, affecting the immunological environment [20].

### *2.4. T Cell Subsets and Infertility*

Early in pregnancy, T cells represent 10–20% of uterine immune cells, predominantly CD8+ T cells and FOXP3+ cells, and Th1 cells are moderately elevated, whereas Th2 and Th17 cells are not enriched. Treg cells suppress inflammation and allow successful embryo implantation [18,21]. Treg cell-mediated tolerance arises in the preimplantation phase of early pregnancy. It depends on interactions between maternal, paternal, and concept-derived signals at the mucosal surface of the uterine endometrium. RIF is associated with insufficient Treg cells in the uterine mucosa or decidual lining [22–24].

Since the immune mechanisms behind implantation failure, recurrent fetal loss, and overall IVF failure are numerous and include many immune cells, receptors, and cytokines, the treatment options for immunomodulation are also numerous. In Figure 1, we present the immune mechanisms in the decidua following the implantation of a zygote.

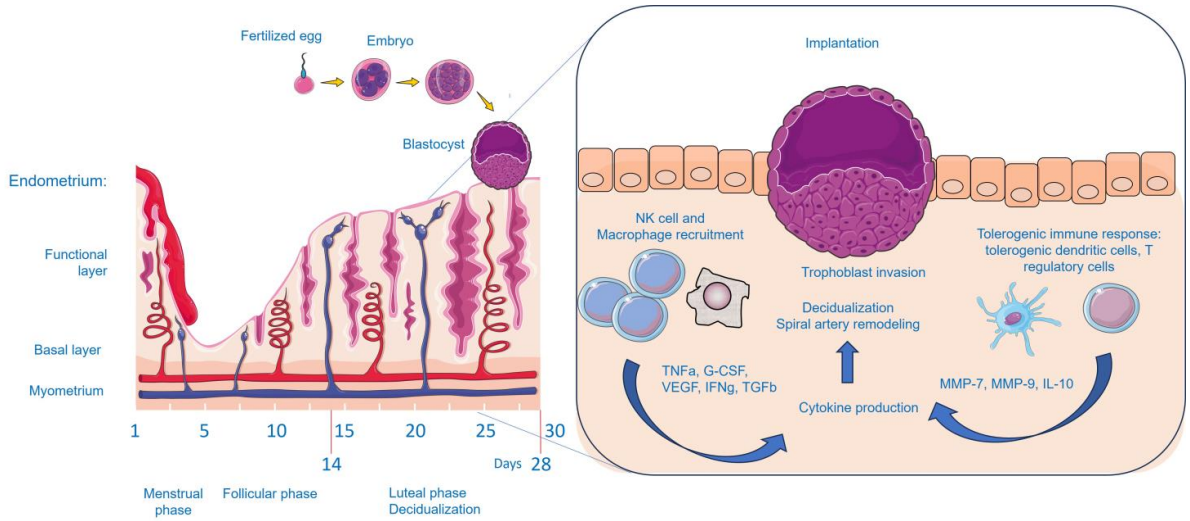


**Figure 1.** Immune mechanisms in the uterus during zygote implantation. Immunotolerance in the decidua during implantation can be achieved by synchronous and regulated processes, such as

selective homing of immune cells to the feto-maternal site, regulated proliferation, and predominant differentiation into a regulatory type of immune cells with the overall goal of the immune responses switching to tolerance, which is a prerequisite for a successful pregnancy. Possibly, dysregulated immune responses and an imbalanced cytokine network may be related to infertility, implantation failures after IVF, and recurrent pregnancy losses. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons BY 4.0 (https://creativecommons.org/licenses/by/4.0/, last accessed on 20 August 2024).

## 3. Intralipid Mechanisms of Action and Effects on Immunity

### *3.1. General Information and Molecular Mechanisms of Action Exerted by Intralipid*

Intralipid is a sterile lipid emulsion of polyunsaturated fatty acids derived from soya bean oil and egg yolk phospholipids used for parenteral nutritional support. It may have immunomodulatory, anti-inflammatory, and antioxidative properties [25]. Since intralipid is a synthetic product traditionally used as a dietary supplement for individuals unable to eat orally because of its fat content, it can nourish these patients by offering energy and necessary fatty acids [26]. The active ingredient in intralipid is pure soybean oil combined with refined egg phospholipids in the following formula: 10% soybean oil, 1.2% egg yolk phospholipids, 2.25% glycerine, and water [26]. However, intralipid has physiologic activities, including immunological function, in addition to its nutritional purpose as an energy source [27]. Additionally, more studies are needed to support the routine use of intralipid in IVF, especially on the safety and efficacy [9].

Although the exact chemical process by which intralipid inhibits NK function is unknown, a generalization based on existing knowledge regarding fatty acids can be made. Intralipid molecules function as ligands for the G-protein-coupled receptor, which in turn activates the nuclear factor kappa-light-chain-enhancer of the activated B cell (NFkB)-related cyclic adenosine monophosphate (cAMP) signaling cascade. Ultimately, the NFkB pathway modifies DNA transcription and regulates critical immune responses [28].

### *3.2. Effects of Intralipid on NK Cells, NK Cell Activity, and Other Immune Cells*

Some studies have shown the immunological effects of intralipid [29–31]. However, the impact of intralipid in pregnancy has yet to be thoroughly understood, particularly in women with RPL. Although all the immunological mechanisms of intralipid are not fully understood, multiple investigations have shown that its active component, soybean oil, inhibits the cytotoxic activity of natural killer (NK) cells [32,33]. Furthermore, its key component, soybean oil, can inhibit pro-inflammatory cells, such as Th1 cells [29,32], as well as pro-inflammatory cytokine production [28,34].

Roussev et al. [30,31] observed that intralipid suppressed NK cell cytotoxicity and that this effect is almost identical to IVIG infusion. A modest degree of NK cell cytotoxicity is maintained after the administration of intralipid. Furthermore, the rates of live birth after IVIG therapy and intralipid treatment were comparable [35]. These findings confirmed the authors' previous discoveries with K562 cells. However, intralipid did not directly reduce NK cell cytotoxicity, suggesting that the action of intralipid on NK cells is indirect. Furthermore, there have been no data on the expression of NK cell receptors such as NKp46 or the proportion of NK22 cells in women with infertility treated with IVIGs or intralipid [30,31].

Therefore, intralipid would be recommended for those patients with increased NK cells to improve live birth rates. Indeed, uterine biopsies from women who have experienced RPL show increased NK cells. It has also been noted that elevated peripheral blood NK cells increase the risk of RPL, especially in women with a history of miscarriage, compared to those without a history of miscarriage [28,35]. Therefore, these are possible indications for intralipid infusions. However, intralipid is unlikely to improve live birth rates in women with fetal chromosomal defects or anatomic, hormonal, or thrombotic risk factors contributing to their pregnancy losses. As a result, to find the person most likely to respond to intralipid, documentation of an immunologic risk factor and the lack of non-immune

risk factors would be required before the decision for therapy [35]. However, NK cells, macrophages, and other innate immune cells that benefit pregnancy may respond to intralipid therapy, according to studies by Foyle et al. [36].

### *3.3. Effects of Intralipid on Cytokine Levels*

After intralipid infusion, several cytokines were elevated in the uterine endometrium, which may affect embryo implantation. Higher plasma C-reactive protein (CRP) levels also accompanied this inflammatory state in patients who became pregnant and underwent IVF compared to those that were not pregnant [36]. Although the increase in plasma cytokines after intralipid treatment was more significant than the expected increase due to cycle-related fluctuations, it is impossible to attribute the elevated plasma cytokines conclusively to intralipid therapy.

The most remarkable changes after intralipid were observed for interleukin (IL)-6 and CXC motif chemokine ligand 8 (CXCL8), which play essential roles in uterine spiral artery remodeling to facilitate early placental development. The increase in tumor necrosis factor (TNF) and C-C motif chemokine ligand 2 (CCL2) may be due to increased production by Th17 cells and NK cells. Since granulocyte-colony-stimulating factor (G-CSF) is a cytokine that promotes blastocyst survival and implantation competence, it may have therapeutic potential in women with RIF [36]. A vascular endothelial growth factor (VEGF) is elevated in the endometrial tissue of women who later have successful pregnancy outcomes and is reduced in women with RIF. Intralipid therapy boosts plasma VEGF and CCL2 levels, although this was outside the context of IVF and pregnancy.

Most of the known immune mechanisms of intralipid are presented in Figure 2.

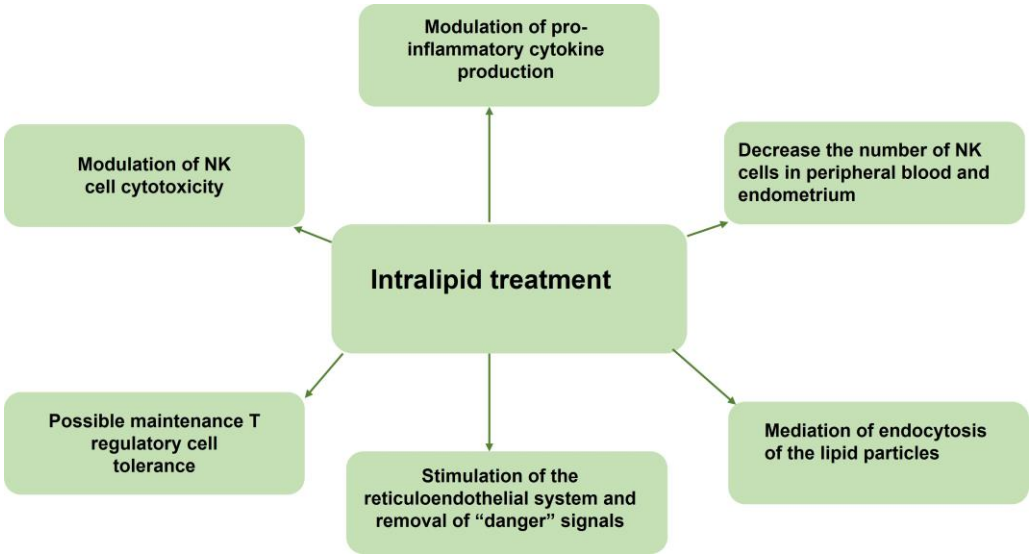


**Figure 2.** Common effects of intralipid treatment on immune mechanisms.

## 4. Intralipid for Infertility Treatment

Successful pregnancy following intralipid injection was described a decade ago [37] and has been studied further in clinical trials. In women with increased NK cell activity caused by an autoimmune etiology (antiphospholipid antibodies and/or antithyroid antibodies), intralipid can be administered 7–10 days before embryo transfer and again after a positive pregnancy test [25,27,38].

### *4.1. Intralipid Effects on T Cells*

Foyle et al. examined the effects of intralipid on circulating T cells in women undergoing assisted reproduction treatment. The study found no increase in Treg cells, no substantial shift in the balance of CD4+ or CD8+ regulatory to conventional T cells, and no indication of an altered phenotype in Treg cells [36]. The study participants included

14 women with a mean age of 35.8 years, a BMI of 25.7, and a total number of embryos transferred in prior assisted reproduction technology (ART) cycles. Intralipid infusions did not alter the relative abundance of white blood cells in the peripheral blood. A slight reduction in CD4+ T cells amongst total CD3+ T cells was present after intralipid treatment, although neither conventional CD4+FOXP3 T cells nor CD4+CD25+CD127$^{low}$FOXP3+ Treg cells were significantly changed [36]. However, the proportion of CD8+ T cells amongst CD3+ T cells was proportionally increased after intralipid infusion. Intralipid treatment was also associated with increased plasma levels of several cytokines and chemokines (as described above) but not with implantation success or later live birth [36].

### *4.2. Intralipid Effects on NK Cells*

While it has been suggested that intralipid may suppress aberrant uNK cell populations and lower NK cell cytotoxic activity in vitro [30], fewer research studies have indicated that intralipid may reduce NK cell frequency in peripheral blood [39]. Furthermore, while increased NK cell activity is frequently associated with pregnancy failure, the relevance of this is debatable [40]. Based on previous research on the effects of lipid emulsions in different therapeutic contexts, there is a biological reason to believe that intralipid may modify the quantity or phenotype of T cells critical for uterine receptivity [29,41,42], as well as other lymphocyte subsets, such as NK cells [30–32]. Roussev et al. demonstrated that infusions of 2 mL (9 mg/mL) or 4 mL (18 mg/mL) of intralipid 20% diluted in 250 mL saline can suppress the NK cell activity within the first week. Furthermore, the normalization of NK cell activity took between 6 and 9 weeks [30].

### *4.3. Comparison of Intralipid and Other Immunomodulators for Reproductive Failure*

Many studies compared the clinical effectiveness of intralipid to other immunomodulators. There were no differences in pregnancy outcomes between women who had a history of reproductive failure and higher NK cell cytotoxicity treated with intralipid and those treated with IVIGs [31]. In addition, intralipid is nearly ten times less expensive than IVIGs, it is not a blood product, and it has no notable adverse effects [32]. Both animal and human studies show that intravenously administered intralipid may improve implantation and pregnancy maintenance when the patient has abnormal NK cell levels or function [43].

Furthermore, Meng et al., in their prospective, randomized clinical trial, revealed that intralipid could be used as an alternate treatment to IVIGs for treating unexplained recurrent spontaneous abortion (referred to as the loss of three or more successive pregnancies before the 20th week of gestation despite normal findings on regular screening procedures) [39]. The authors proposed that intralipid works by modulating NK cell activity and increasing trophoblast invasion. In some of the cases with unexplained recurrent spontaneous abortion, we supposed immune dysfunction; however, elevated NK cells and cytotoxicity are more researched and validated as contributors to infertility [39]. Similarly, Ehrlich et al. (2019), in their exploratory, retrospective cohort research, focused on the pregnancy outcomes and adverse events related to intralipid usage in 93 women. One patient with a history of seizures experienced a “pre-seizure, flushing” feeling. Asymmetrical intrauterine growth restriction was observed in one pregnancy. Other adverse effects during pregnancy were not recorded [44].

A systematic review and meta-analysis by Han et al. (2021) included five randomized clinical trials with 840 patients to evaluate whether intralipid administration improved the outcomes of IVF [45]. The results suggested that intralipid administration may benefit women undergoing IVF, especially those who have experienced repeated implantation failure or recurrent spontaneous abortion [45]. Furthermore, intralipid treatment substantially enhanced the clinical pregnancy rate (risk ratio [RR], 1.48; 95% confidence interval [CI], 1.23–1.79), continuing pregnancy rate (RR, 1.82; 95% CI, 1.31–2.53), and live birth rate (RR, 1.85; 95% CI, 1.44–2.38) when compared to the control group. However, intralipid treatment did not change the miscarriage rate (RR, 0.75; 95% CI, 0.48–1.17) [33]. Han et al. concluded that intralipid treatment might enhance IVF outcomes, particularly in women with previous

miscarriages [45]. However, due to several limitations of this analysis, intralipid in women undergoing IVF should be taken with caution, and these findings need to be validated in more extensive, well-designed investigations.

Another systematic review and meta-analysis was conducted by Rimmer et al. (2021) on five randomized trials reporting on 843 women. All trials employed a 20% intralipid solution during embryo transfer as opposed to a saline infusion or no intervention (routine care) [46]. Compared to no intervention, the intralipid group had a greater likelihood of clinical pregnancies (172 vs. 119, RR 1.55, 95% CI 1.16–2.07) and live births (132 vs. 73, RR 1.83, 95% CI 1.42–2.35) [46]. It is also thought that intralipid may minimize the incidence of placenta-mediated pregnancy problems (e.g., preeclampsia), which are more frequent in women using ART, as it promotes implantation and placentation [47]. A study by El-Gegawy explored the effects of intralipid infusion during pregnancy as a complementary treatment for antiphospholipid syndrome to avoid complications in 105 pregnant patients with promising results [48]. Kumar et al. also confirmed that intralipid administration enhanced IVF pregnancy rates and lowered the miscarriage risk in some patient groups in the study. The authors selected and evaluated 12 trials, demonstrating that intravenous intralipid therapy improves implantation, pregnancy, and live birth rates while decreasing miscarriage rates [37]. In addition, this study found evidence to support the use of intralipid in select individuals when traditional therapies have failed. A comprehensive overview of studies [35,39,44,49–56] exploring the efficacy and safety of intralipid is shown in Table 1.

Allahbadia stated that intralipid has been the favorite gynecological approach for immunotherapy since 2015 [25]. Comparative studies of immunomodulatory therapy for reproductive failure so far showed no differences observed between the intralipid-treated and IVIG-treated pregnancy outcomes of women having a history of reproductive failure and higher NK cell cytotoxicity [30,31,35,43,57]. A side-by-side study revealed that the currently employed IVIG or intralipid therapies were less effective than a synthetic preimplantation factor (sPIF) at inhibiting NK cell toxicity at a lower dose [58].

Additionally, soluble human leukocyte antigen (sHLA)-G, intralipid, and IVIGs were comparatively tested to determine how well they could reduce the cytotoxicity of NK cells in vitro. It was shown that sHLA-G suppressed NK cell cytotoxicity by 39.9 ± 5.0%, intralipid by 39.8 ± 6.2%, and IVIGs by 38.9 ± 5.4%, concluding that the three therapeutic approaches inhibited NK cell activity equally well in in vitro assays [31]. Nevertheless, as we pointed out, one of the significant advantages of intralipid is its global availability and affordable price, unlike IVIGs [25]. In Figure 3, we present the treatment approaches in reproductive medicine and the place that intralipid has there.

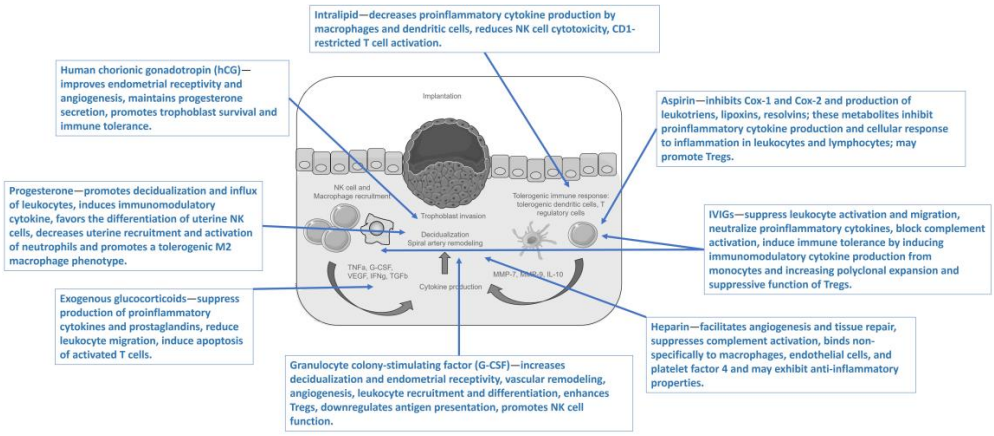


**Figure 3.** Immunomodulatory treatment in reproductive failure: targets and drugs. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons BY 4.0 (https://creativecommons.org/licenses/by/4.0/, last accessed on 20 August 2024).

**Table 1.** Studies exploring the efficacy and safety of intralipid for reproductive failure.

| Study Design | Indications | Subjects | Type of Intervention | Medication | Outcomes | Adverse Effects | Ref. |
|---|---|---|---|---|---|---|---|
| Matched control | History of RPL or RIF | 10 patients aged 40–42 years and 10 controls | Intralipid vs. no treatment of the controls | Intralipid 4 mL diluted at 20% in 100 mL saline, infusion over 1 h | CPR; LBR; MR—no significant difference | N/A | Check and Check (2016) [49] |
| RCT | ≥3 unexplained miscarriages before 12th gestational week; peripheral NK cells >20% | 76 patients vs. 78 controls | Intralipid vs. IVIGs | 20% intralipid in in 250 mL saline, infusion over 2 h vs. 25 g IVIG infusion over 8 h | CPR; LBR—no significant difference | No adverse effects | Meng et al. (2016) [39] |
| Cohort study | ≥3 recurrent miscarriages before 12th gestational week and/or ≥3 implantation failures of ≥2 good embryo transfers; absence of any cause of RPL or RIF | 26 patents vs. 36 controls | Intralipid vs. placebo | Intralipid infusion + low-dose aspirin; prednisolone (10 mg/day); progesterone; vitamin D | CPR; LBR—significant improvement | N/A | Placais et al. (2020) [50] |
| Cohort Study | ≥3 unexplained miscarriages or infertility; peripheral NK cells >19% | 127 patients vs. 20 controls | Intralipid vs. placebo | 4 mL intralipid diluted at 20% in 250 mL saline, infusion over 90–120 min | CPR; LBR—no significant difference | Reduced side effects | Martini et al. (2018) [51] |
| Cohort study | History of RIF and/or RPL | 134 patients vs. 134 controls | Intralipid vs. no treatment | 20% intralipid + Prednisolone 15–25 mg; Omega 3.3 g; B complex; vitamin D3; LMWH | CPR; IR; MR—significant improvement | N/A | Harrity et al. (2018) [52] |
| Cohort study | History of unexplained infertility, RIF, RPL | 200 patients vs. 242 controls | Intralipid vs. IVIG | N/A | CPR; LBR—no significant difference | N/A | Coulam and Acacio (2012) [35] |
| Non-randomized study | ≥3 implantation failures with elevated TH1:TH2 cytokine ratios | 50 patients vs. 46 controls | Intralipid vs. no treatment | 20% intralipid | CPR—significant improvement | N/A | Ndukwe (2011) [37] |
| RCT | Failure to achieve pregnancy after 2–6 ICSI cycles with the transfer of ≥10 high-grade embryos | 101 vs. 102 patients | Intralipid vs. no treatment | 20% intralipid | CPR; IR; LBR—a significant improvement | N/A | El-Khayat and Sadek (2015) [53] |
| RCT | Age < 42 years with BMI < 30 kg/m$^2$; ≥3 RIFs undergoing ICSI cycles | 71 patients vs. 71 controls | Intralipid vs. no treatment | 100 mL intralipid diluted at 20% in 500 mL saline) infusion over 150 min | CPR; LBR—significant improvement | N/A | Al-Zebeidi et al. (2020) [54] |
| RCT | Age group 20–40 years; with primary infertility undergoing non-donor oocyte IVF/ICSI with at least one previous implantation failure | 52 patients vs. 50 controls | Intralipid vs. saline | 4 mL intralipid diluted at 20% in 250 mL saline, infusion | Biochemical pregnancy rate; CPR; LBR; take-home baby rate—significant improvement | N/A | Singh et al. (2019) [55] |

**Table 1.** *Cont.*

| Study Design | Indications | Subjects | Type of Intervention | Medication | Outcomes | Adverse Effects | Ref. |
|---|---|---|---|---|---|---|---|
| Cohort study | History of repeated unsuccessful IVF cycles and pre-viable pregnancy loss | 93 patients vs. 651 controls | Intralipid vs. no treatment | 100 mL intralipid diluted at 20% in 500 mL saline, infusion over 3–4 h + prednisolone; LMWH; aspirin; heparin | CPR; LBR—no significant difference | Very low rate of adverse effects | Ehrlich et al. (2019) [44] |
| RCT | Women with a history of recurrent implantation failure after IFV/ICSI | 97 subjects | Intralipid vs. placebo | 100 mL intralipid diluted at 20% 6–7 days before embryo transfer + repeated dose in case of positive pregnancy test | Live birth, CPR | May increase the risk of congenital malformations | Gamaleldin et al. 2018 [56] |

CPR—clinical pregnancy rate; IR—implantation rate; MR—miscarriage rate; LBR—live birth rate, IVF—in vitro fertilization, ICSI—intracytoplasmic sperm injection, RCT—randomized controlled trial.

## 5. Controversies Regarding Targeting NK Cells and NK Cell Activity by Intralipid

Despite the reporting that there is no evidence linking the use of intralipid to unfavorable reproductive outcomes, there is a growing body of published data, randomized controlled trials, and systematic reviews that address this topic. Immunological maladaptation is an essential factor in some obstetric problems, including pregnancy-induced hypertension, preeclampsia, and intrauterine growth restriction [59]. While it is well known that healthy endometrial immune function—precisely, the presence of uNK cells—is necessary for implantation and the development of the first few months of pregnancy [60], there is still much to learn about the effects of variations in leukocyte counts.

Also, considerable criticism is being raised about using peripheral NK and NKT cell numbers as a marker of their increase in the uterus because of external dynamics and because they are prone to fluctuations, which influence blood values, so they tend to lack scientific credibility. However, peripheral NK cells are reported to be elevated in women with RPL [61–63], and peripheral blood testing is routinely used. According to Martini et al. [51], intralipid has fewer adverse effects and a lower patient risk than the alternatives, which makes it a safer and more widely accepted option. The deficient number of adverse events observed in pregnancy outcomes after intralipid usage suggests that it is a safe medication to use in the RIF/RPL population [39,44].

Reproductive immunology is becoming a very popular field, and in the near future, personalized therapy and diagnostic testing might be feasible. This is significant because individuals with RPL comprise a susceptible group that will seek out experimental treatments in the event that a live birth is possible [64]. However, a complex sequence of events is needed for successful trophoblast invasion, vascular remodeling, and tolerance induction to an antigenically different fetus during the immune system switching to pregnancy mode. Reproductive failure on the part of the individuals is most certainly caused by a dysregulated immune response, but there are many different ways that this can happen; therefore, immune treatment needs to be tailored to the particular condition. Regretfully, immunological modulation has produced unsatisfactory clinical outcomes thus far [64].

## 6. Conclusions

In conclusion, the evidence so far justifies the use of intralipid infusion in women with a history of reproductive failure. Systematic reviews and meta-analyses confirmed the beneficial immunological effects of intralipid and its favorable safety profile. Intralipid therapy improves implantation and live birth rates while decreasing miscarriage rates. In addition, intralipid may be used in select individuals when traditional treatments have failed. However, intralipid infusions should be administered to a subset of patients where immunological risk factors are present, traditional therapies have not worked, and

regular laboratory results are unremarkable. Further research is necessary to identify the individuals who could benefit from the presence of aberrant uterine uNK cells as a target marker and for the routine use of intralipid in IVF.

**Author Contributions:** Conceptualization, T.V. and G.N.; investigation, L.T.; writing—original draft preparation, T.V.; writing—review and editing, G.N. and L.T.; visualization, T.V.; supervision, T.V.; project administration, T.V. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No BG-RRP-2.004-0008.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** Parts of Figures 1 and 3 were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons BY 4.0 (https://creativecommons.org/licenses/by/4.0/, last accessed on 20 August 2024).

**Conflicts of Interest:** The authors declare no conflicts of interest.

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