Original Article

Evaluating the Utility of Intralipid Infusion to Improve Live Birth Rates in Patients with Recurrent Pregnancy Loss or Recurrent Implantation Failure

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Abstract

Context: Intralipid is used to improve clinical outcomes in patients with recurrent pregnancy loss (RPL) or recurrent implantation failure (RIF) with elevated natural killer (NK) cells. Data supporting this practice is conflicting but suggestive of minimal benefit. **Aims:** The aims of this study are to determine if intralipid infusion improves live birth rates and if is a cost-effective therapy in the RPL/RIF population. **Settings and Design:** This was a large REI private practice, retrospective cohort study. **Subjects and Methods:** Charts of 127 patients who received intralipid from 2012 to 2015 were reviewed and compared to historical control data. *T*-tests and Chi-square analyses evaluated demographics and cycle statistics. Chi-square analyses assessed impact on clinical pregnancy and live birth rates. Cost analysis was performed from societal perspective with a one-way sensitivity analysis. **Results:** Patients with live births were noted to have a higher average number of previous live births and were more likely to have had a frozen embryo transfer in the intralipid cycle in comparison to those with unsuccessful pregnancy outcomes. Neither clinical pregnancy nor live birth rates were significantly improved from baseline rates quoted in the literature (P = 0.12 and 0.80, respectively). Intralipid increased costs by $681 per live birth. If live birth rates were >40% using intralipid and <51% without intervention, neither strategy was favored. **Conclusions:** Intralipid does not improve live birth rates and is not cost-effective for patients with RIF or RPL and elevated NK cells. This study supports the growing literature demonstrating the minimal benefit of screening for and treating elevated peripheral NK cells.

Keywords: *Intralipid, natural killer cells, recurrent miscarriage, recurrent pregnancy loss*

Introduction

Recurrent pregnancy loss (RPL) has historically been described as three or more miscarriages before 20 weeks of gestation.[1] The American Society for Reproductive Medicine more recently redefined this phenomenon as having two or more failed pregnancies which do not necessarily have to be consecutive.[2] There is no consensus definition for recurrent implantation failure (RIF); however, it has been cited to include three or more failed treatment cycles as well as failure to achieve a clinical pregnancy after transfer of at least four good quality embryos over three fresh or frozen cycles.[3,4] The diagnosis of RPL and RIF are distinctly different from infertility, particularly in regard to the causes, warranted investigations, and the emotional strain that it places on patients. Although the etiologies of RPL and RIF can be discovered and remedied in some, approximately 50% of cases have no identifiable

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explanation leaving patients desperate for answers and at times seeking alternative therapies.[5]

There have been studies proposing an immunological explanation for the RPL/RIF phenomenon, mainly in the absence of an identifiable cause. Human self-recognition of an embryo or fetus is poorly understood, and it has been argued that alterations in this process may contribute to the failure to achieve a clinical pregnancy. Various biochemical factors have been implicated including local inflammatory mediators, human leukocyte antigens, and elevated levels of circulating natural killer (NK) cells.[6] Targeted immune modulators have been proposed to remedy this problem; however, their ability to improve pregnancy outcomes in patients with RPL or RIF has been disputed.[7]

A recent meta-analysis of 20 high-quality trials totaling 1137 women assessed multiple immune therapies including paternal leukocyte immunization, intravenous immune globulin, trophoblast membranes, and third-party donor leukocytes revealed no increase in live birth rates with any intervention when compared with placebo or no treatment.[8] Intralipid, a soybean oil-based lipid emulsion traditionally used for parenteral nutrition, has been less frequently investigated in the treatment of immune-mediated pregnancy failure.

Intralipid has been demonstrated to be effective in decreasing NK cell activation and production of proinflammatory cytokines.[9-11] A review by Coulam and Acacio examined pregnancy outcomes in 200 RPL/RIF patients with increased NK cell activity and quoted a 61% live birth rate following treatment with intralipid therapy which did not significantly differ from that achieved with intravenous immunoglobulin (IVIG).[12] A recent double-blind randomized control trial of 296 women with secondary infertility, recurrent miscarriage, and elevated NK cells showed no increase in clinical pregnancy rates in the 144 women who were assigned to receive intralipid therapy.[13] A prospective trial by Check and Check showed similar results but in a significantly smaller and more specialized cohort of women.[14] A review by Shreeve *et al*. concluded that the current evidence promoting intralipid therapy is not substantial enough to support its routine use for patients with RPL/RIF.[15] Even with a larger quantity of data in contradiction of the routine use of intralipid, its impact on live birth rate has not been consistently evaluated.

Based on the negligible or limited benefit that is suggested by these studies, it is appropriate to question whether it is reasonable for both patients and institutions to continue paying for these therapies. IVIG is a notoriously expensive intervention with costs ranging $7000 to $14,000 for a single infusion.[16] In addition, IVIG poses risks of anaphylaxis and a low but plausible risk of infection transmission.[17] Intralipid has been promoted as a less expensive, safer, and equivalent alternative to IVIG; however, there are no cost analyses to validate this idea.[12,18]

Despite the data demonstrating minimal success, IVF centers continue to offer this treatment. We explored the benefit of intralipid for RPL/RIF patients at a large REI practice. The goals of this study were to examine whether pregnancy outcomes, specifically live birth rates, were improved when patients diagnosed with RPL or RIF and elevated NK cells received intralipid therapy. If benefit occurred, our study sought to elucidate if it was a cost-effective intervention.

Subjects and Methods

A retrospective cohort study was performed at a large clinical infertility private practice. A total of 127 patients were identified who received intralipid infusion from 2012 to 2015. Patients were screened for use of intralipid therapy based on elevated peripheral NK cells and an identified history of unexplained RPL or RIF by the treating provider. IRB approval was obtained.

The majority of patients conceived using ART with autologous embryos. Patient demographics and cycle statistics were collected. These included age at cycle start, body mass index (BMI), antimullerian hormone level (AMH), number of prior *in vitro* fertilization (IVF) cycles, cycle type (including fresh embryo transfer, frozen embryo transfer (FET), or intrauterine insemination (IUI), estradiol (E2) level on day of HCG trigger, total number of oocytes retrieved, number of metaphase 2 (M2) oocytes retrieved, number of embryos transferred, and overall cycle outcome. The patients' pregnancy and fertility histories were also collected including their total number of pregnancies (gravidity) and number of previous live births, miscarriages, clinical pregnancy losses, biochemical losses, second-trimester miscarriages, ectopic pregnancies, and number of failed implantations and IUIs.

Levels of peripheral NK cells were measured using flow cytometry with LabCorp "Natural Killer Cell Surface Antigen ($CD3^-$ $CD56^+$ marker analysis, Test No. 505016)." Included in the results of this assay was the percentage of $CD3^-$ $CD56^+$ NK cells, absolute $CD3^-$ $CD56^+$ NK cells as well as an absolute lymphocyte count. For the 127 patients in this study, this test was performed within at least 2 weeks before the intervention and monitored weekly following the infusion. A value of more than 19% was considered elevated based on adult reference ranges established by

the laboratory. The goal for treatment was to maintain NK cell levels <10%.[19]

The infusion contained 4 mL (20%) intralipid solution injected into 250 mL (0.9%) normal saline. The infusions were generally administered 7–10 days before embryo transfer or insemination, and if the patient became pregnant, it was repeated at approximately 6 weeks' gestation and again at approximately 10 weeks' gestation. The infusion was initially run at 50 mL/h, and if tolerated by the patient, the rate was increased to be completed over 90–120 min. Intralipid was contraindicated women with disturbances of normal fat metabolism such as pathologic hyperlipidemia, lipoid nephrosis, impaired kidney function, or acute pancreatitis if accompanied by hyperlipidemia.

Statistical power calculation was performed assuming a 40% baseline live birth rate for a RPL population before any intervention. This necessitated the recruitment of 271 patients to demonstrate a 10% increase in live birth rate and 1200 patients to demonstrate a 5% increase in live birth rate. *T*-tests were used for continuous variables and Chi-square tests for categorical variables when comparing demographics and cycle parameters within the intralipid cohort stratified by pregnancy outcome as well as between the intralipid cohort and the historical cohort in the article by Tang *et al*. Chi-square analyses were also used to evaluate the impact of intralipid on clinical pregnancy and live birth rates utilizing a 70% clinical pregnancy rate and a 40% baseline live birth rate in an untreated RPL population with elevated NK cells.[20]

Cost analysis was performed from a societal perspective, and the live birth rate was the measured effectiveness. Published IVF costs, estimated intralipid drug costs and internal staff costs were utilized.[21-23] Only direct costs were included. Specifically, this included cost of supplies estimated at $5.31 per infusion (including IV solution and tubing) and 2-h nursing costs estimated at $71.31 per infusion. The cost of the intralipid drug was approximately $56 per infusion.

Base-case ranges for live birth rates in a RPL population were estimated from the literature and our gathered data.[20] When ranges for variables were not available in the literature, they were generated from half to twice of the base-case value. Tree Age software was used for this analysis. One-way sensitivity analysis was conducted to test the robustness of the model.

Results

Pregnancy outcomes for the intralipid cohort were collected and are listed in Table 1. Of the 127 patients, 51 did not achieve pregnancy. Of the patients that achieved pregnancy, 10 were biochemical pregnancies, one was an ectopic pregnancy, 16 had first trimester losses, two had second-trimester losses, and 47 experienced live births.

We compared the age, BMI and pregnancy histories of the intralipid cohort (n = 127) to a historical cohort (n = 20) which is relayed in Table 2. Age (P < 0.001), number of previous miscarriages (P < 0.001), number of previous clinical pregnancy losses (P < 0.001), number of biochemical losses (P < 0.001), and percentage of patients with at least one prior live birth (P = 0.046) were all statistically different between the two groups.

Demographic information and cycle statistics were compared between those with live births and those with all other outcomes (unsuccessful outcomes) as outlined in Table 3. The average age was neither statistically significant between the two groups (36.1 ± 5.1 years for live birth group versus 35.7 ± 4.2 years for unsuccessful group, P = 0.61) nor was average BMI (25.6 ± 5.6 vs. 26.2 ± 6.8 kg/m^2, P = 0.57). The two groups had comparable pretreatment AMH levels (3.4 ± 4.2 vs. 2.7 ± 3.0 ng/mL, P = 0.39), and average E2 levels on day of human chorionic gonadotropin trigger (2964 ± 1237.9 vs. 2219.6 ± 1255.3 pg/mL, P = 0.22). The two groups additionally had similar average numbers of prior IVF cycles (1.6 ± 1.1 vs. 1.7 ± 1.3, P = 0.86). There was a statistically higher number of oocytes retrieved (16.3 ± 10 vs. 9.8 ± 5.7, P = 0.025) as well as a higher number of M2 oocytes obtained in the live birth group when compared to the unsuccessful outcome group (11.8 ± 6.1 vs. 7.8 ± 4.3, P = 0.05). The number of embryos transferred during the cycle of study was not statistically different between the two groups (1.6 ± 0.60 vs. 1.7 ± 0.70, P = 0.37). The majority of patients in both groups underwent FET during the cycle of study (83% in live birth group vs. 51% in unsuccessful group); however, a smaller percentage of patients underwent IUI in the live birth group (2% in live birth group vs. 7.5% in unsuccessful group).

Table 1: Pregnancy outcomes for the 127 study patients who received intralipid therapy

Pregnancy outcome	Number of patients
Not pregnant	51
Biochemical	10
Ectopic	1
First-trimester loss	16
Second-trimester loss	2
Live birth	47
Total	127

RPL=Recurrent pregnancy loss, RIF=Recurrent implantation failure, NK=Natural killer

Table 2: Demographics and pregnancy histories of the intralipid cohort compared to a historical control population

	Intralipid cohort (*n*=127)	Historical control cohort (Tang *et al.*) (*n*=20)	*P* (statistical significance ≤0.05)
Demographics			
Age (years)	35.9±4.5	33 (27-39)	<0.001
BMI (kg/m^2)	25.8±6.1	26 (21-32)	NS (0.62)
Pregnancy history			
Number of previous miscarriages	1.4±1.4	5 (3-15)	<0.001
Number of previous clinical pregnancy losses	0.93±1.1	1.35 (0-5)	<0.001
Number of previous biochemical losses	0.5±0.78	3.8 (1-10)	<0.001
Number of patients with at least one previous live birth (%)	62/127 (48.8)	3/20 (15)	0.046
Number of patients with at least one previous second-trimester miscarriage (%)	5/127 (3.9)	2/20 (10)	NS (0.23)
Number of patients with at least one prior ectopic pregnancy (%)	12/127 (9.4)	2/20 (10)	NS (0.94)

The control cohort contained patients with elevated NK cells that received no intervention. The article by Tang *et al*. that supplied the control cohort reported ranges instead of standard deviations for age, BMI, number of previous miscarriages, number of previous clinical pregnancy losses and number of previous biochemical losses. NS=Not significant, BMI=Body mass index, NK=Natural killer

Pregnancy histories differed between those with live births and those with unsuccessful outcomes in number of previous live births (1.3 ± 0.63 vs. 0.31 ± 0.63, $P < 0.001$), number of total previous miscarriages (1.1 ± 1.2 vs. 1.7 ± 1.5, $P = 0.03$), and number of previous clinical pregnancy losses (0.64 ± 0.93 vs. 1.1 ± 1.2, $P = 0.04$).

Chi-squared analyses comparing pregnancy outcomes in those who received intralipid are displayed in Table 4. Intralipid administration did not result in a significantly higher number of clinical pregnancies when compared to baseline clinical pregnancy rate in the control population ($P = 0.12$). In addition, the intralipid cohort did not have a significantly higher number of live births when compared to the control population ($P = 0.80$). Again, a 70% clinical pregnancy rate and a 40% live birth rate were presumed using the previously published data.[20]

Cost-benefit analysis of the administration of intralipid therapy is presented in Table 5. It demonstrated that the administration of intralipid infusion increased overall treatment costs by $681 per live birth. Sensitivity analysis demonstrated if live birth rates were above 40% for the intralipid group and <51% for those without intervention (standard care group) that neither strategy was superior to the other.

Discussion

Our study observed no improvement in live birth rates when intralipid therapy was administered to patients with a history of RPL or RIF with elevated levels of NK cells. These results echo findings from prior studies which also concluded that the use of intralipid in the RPL/RIF population failed to have a clinically significant impact.[13-15] Based on these results, our practice no longer screens peripheral NK cells in RIF/RPL patients.

Our results noted that higher numbers of mature oocytes and total number of oocytes retrieved in patients who experienced live births. These findings are consistent with prior research that shows an association between live birth rates and greater ovarian response.[24-27] In addition, patients in the live birth cohort had significantly higher numbers of previous live births and significantly lower numbers of prior miscarriages including clinical pregnancy losses. Alongside these findings, there were a significantly higher number of RPL patients in the unsuccessful group. Although this unequal distribution may slightly impair the impact of our findings, the above stands to suggest a mechanistic difference between RPL and RIF and that they should analyzed as separate conditions in future studies examining targeted therapies.

The majority of patients in this study conceived through IVF with fresh orFET. There was a small subset of patients that underwent IUI who were included due to a diagnosis RPL. Notably, there was a larger percentage of patients that underwent FET in the live birth group (83%) when compared to the unsuccessful outcomes group (51%). Recent studies have remarked on the difference in endometrial receptivity between fresh and frozen transfer cycles and demonstrated superior IVF outcomes in those patients who underwent embryo cryopreservation followed by FET in the subsequent cycle.[28,29] Admittedly, if this is the case, this could provide a potential explanation for the distribution

Table 3: Demographics, pregnancy histories, and cycle statistics of women with live births compared to those with unsuccessful pregnancy outcomes

	Patients with live births (*n*=47)	Patients with unsuccessful outcomes (not pregnant, biochemical, ectopic, first-trimester loss, second-trimester loss) (*n*=80)	*P* (statistical significance ≤0.05)
Demographics			
Age (years)	36.1±5.1	35.7±4.2	NS (0.61)
BMI (kg/m^2)	26.2±6.8	25.6±5.6	NS (0.57)
Pregnancy history			
Gravidity	2.6±1.4	2.2±1.8	NS (0.28)
Number of previous live births	1.3±0.63	0.31±0.63	$P < 0.001$
Number of previous miscarriages	1.1±1.2	1.7±1.5	0.03
Number of previous clinical pregnancy losses	0.64±0.93	1.1±1.2	0.04
Number of previous biochemical losses	0.44±0.67	0.54±0.84	NS (0.52)
Number of previous second trimester miscarriages	0.02±0.15	0.05±0.22	NS (0.45)
Number of previous ectopic pregnancies	0.09±0.34	0.16±0.49	NS (0.38)
Number of prior failed implantations	1.2±1.2	1.65±1.53	NS (0.09)
Number of prior failed IUIs	1.4±1.4	1.54±2.2	NS (0.71)
Cycle statistics			
AMH (ng/mL)	3.4±4.2	2.7±3.0	NS (0.39)
Number of prior IVF cycles	1.6±1.1	1.7±1.3	NS (0.86)
Estradiol day of trigger (pg/mL)	2964±1237.9	2219.6±1255.3	NS (0.22)
Number of oocytes retrieved	16.3±10.0	9.8±5.7	0.025
Number of M2 oocytes retrieved	11.8±6.1	7.8±4.3	0.05
Number of embryos transferred during Intralipid cycle	1.6±0.60	1.7±0.70	NS (0.37)
Percentage fresh embryo transfer cycle	7/47 (15)	33/80 (41)	0.002
Percentage frozen embryo transfer cycle	39/47 (83)	41/80 (51)	$P<0.001$
Percentage IUI	1/47 (2)	6/80 (7.5)	NS (0.20)
Percentage RIF	32/47 (68)	44/80 (55)	NS (0.15)
Percentage RPL	13/47 (28)	37/80 (46)	0.04

Unsuccessful outcomes included any outcome other than live birth (negative pregnancy tests, biochemical losses, ectopic pregnancies, first-trimester losses, or second-trimester losses). NS=Not signifi cant, BMI=Body mass index, AMH=Antimullerian hormone, IVF=*In vitro* fertilization, M2=Metaphase 2, IUI=Intrauterine insemination, RIF=Recurrent implantation failure, RPL=Recurrent pregnancy loss

Table 4: Chi-squared analysis evaluating the effect of intralipid on clinical pregnancy and live birth rates

	Intralipid cohort (*n*=127) (%)	Historical cohort (Tang *et al.*) (*n*=20) (%)	*P* (statistical significance ≤0.05)
Clinical pregnancy rate	65/127 (51)	14/20 (70)	0.12
Live birth rate	47/127 (37)	8/20 (40)	0.80

Utilizing data from the article by Tang *et al*., a 70% clinical pregnancy rate and a 40% live birth rate were presumed

within the live birth cohort. Although mechanism of achieving pregnancy is important in the interpretation of the results, this factor should not entirely nullify the conclusion that intralipid is not beneficial, as it still failed to demonstrate sizable benefit in a group that over half attempted pregnancy by FET.

A cost analysis review revealed that even at the low cost of the intralipid intervention, there was no benefit to its administration. When the intervention is at such a low cost and still considered cost ineffective in a broad range of theoretical scenarios, it is determined to not be cost-effective, particularly when treatment outcomes are not improved. Furthermore, though indirect costs such as time away from work were not included in the analysis, intralipid was still found to be cost ineffective. Overall, the use of intralipid is not a cost-effective

Table 5: Cost-benefit analysis of intralipid administration

Strategy	Cost	Marginal cost	Effectiveness	Marginal effectiveness	Cost/effectiveness ($)	Marginal cost/effectiveness ($)
No intralipid	10.3K		0.400		25,675.00	
Intralipid	10.3K	0.1K	0.510	0.110	20,284.31	681.82

Cost analysis was performed from a societal perspective and live birth rate was the measured effectiveness. Estimated IVF costs were generated from the literature. The estimated price of the intervention is based on pharmacy charges and internal staff costs. IVF=*In vitro* fertilization

strategy to improve live birth rates in the majority of clinical scenarios.

The novelty in our findings lies in our evaluation of the effect of intralipid therapy on live birth rates which is arguably the only meaningful pregnancy outcome for this patient population. In addition, though promoting its lower cost and enhanced safety profile, no prior studies have examined whether administering intralipid was actually cost-effective for live birth outcomes. The ideal method to examine the impact of this intervention would be a randomized placebo-controlled study, though case–control studies are beneficial to further study disease processes where true randomized trials are not conceivable. Despite the methodology of our study, we believe these results add substance to the current body of literature in opposition to administration of immune therapies.

Our study was indeed limited by its relatively small sample size, and it therefore being underpowered to detect a more subtle increase in the rates of live birth. Furthermore, a notable limitation in this study was the use of historical control data as opposed to age-matched controls with elevated NK cells at the same facility. All patients with NK cell testing at the Fertility Centers of Illinois underwent the intervention and therefore limited the ability to compile a more appropriately matched control group. The historical cohort in the article by Tang *et al*. also had its inadequacies in that there were significant demographic differences including age, number of previous miscarriages, number of previous clinical pregnancy losses, and number of patients with at least one live birth. They also had NK cells measured intrauterine with endometrial biopsy as opposed to peripheral. As discussed further below, there is no standard for NK cell testing which makes it important to interpret these results from a holistic and mechanistic perspective. Nevertheless, this cohort was the best viable option when compiling this data in a retrospective fashion.

Moving forward, it is appropriate to question the relevance of obtaining these immunological markers in the evaluation of patients with otherwise unexplained RPL or RIF, particularly in the absence of a validated target therapy. Prior studies have concluded that elevated levels of circulating or peripheral NK cells have been shown to be present in women with a history of RIF, predictive of women who will miscarry a karyotypically normal pregnancy and associated with miscarriage in a subsequent pregnancy.[30-33] Others have shown no difference in live birth rates in women with elevated peripheral NK cells undergoing IVF when compared to controls.[34]

In addition, there is a striking inconsistency between sources in the cutoff value of NK cells that is considered "elevated" as well as the methodology for measuring NK cells. Roussev *et al*. determined >10% killing activity to be abnormal when NK cells were measured peripherally.[11] Beer *et al*. have suggested that peripheral NK cells are considered elevated when they comprise 12% of all circulating lymphocytes when others have reported a normal range up to 29%.[35,36] Other studies have remarked that peripheral NK cell levels higher than 18% have a high specificity for RPL.[37]

There is also variation across the literature in the techniques used to measure NK cells with uterine and peripheral measurements both being reported. Multiple sources note inconsistencies between peripheral and uterine measurements concluding that one method is not a reliable substitute for the other.[38] Seshadri and Sunkara demonstrated higher peripheral NK cell number and percentage in women with RPL when compared to controls. However, when examining uterine NK cells, there was no difference in patients with RPL when compared to controls.[34] Moffett and Shreeve endorse that uterine NK cells are difficult to measure given their fluctuation throughout the menstrual cycle and stress that though uterine NK cells are presumed to pose a threat to the invading trophoblast, there is no convincing evidence that this is actually the case.[38,39] With this conflicting data and no proven treatment to offer patients at this point, more research is needed before measurement of NK cells should be used as a standardized diagnostic tool.

In summary, these results stand to support current recommendations that advise against routinely offering intralipid therapy for the treatment of RPL or recurrent implantation failure. Despite a more reasonable monetary profile, the cost of administering intralipid still outweighs any theoretical benefit while not producing a measurable increase in the outcome of live birth.

Further, research should be focused on developing a standardized method for the measurement of NK cells and determining appropriate cutoff values, and only after can targeted therapies be adequately evaluated.

Conclusions

Intralipid does not improve live birth rates and is not a cost-effective intervention in patients with recurrent pregnancy loss or recurrent implantation failure. Intralipid should not be routinely offered to these patient populations.

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Conflicts of interest

There are no conflicts of interest.

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