Efficacy of Intralipid Administration to Improve Pregnancy Rates in Unexplained Intracytoplasmic Sperm Injection (ICSI) Failure

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ABSTRACT

Background: In the field of reproductive medicine, one of the most difficult situations is repeated implantation failure (RIF). It is unknown how intralipid directly affects fertility, however it appears to depend on nature killer (NK) cells cytotoxicity being inhibited, maybe through short fatty acids activating PPARγ receptors.

Objectives: The current study aimed to investigate whether intralipid administration could improve the pregnancy outcomes of intracytoplasmic sperm injection (ICSI) cycles in cases with unexplained recurrent implantation failure (RIF).

Patients and methods: A total of 112 frozen embryo transfer cycles with history of unexplained RIF were included in a randomized controlled trial and divided into two equal groups: (A) intralipid-treated group (n = 56), received intravenous infusion of intralipid 100 mL of 20% intralipid diluted in 500 mL of normal saline, administrated approximately 7–10 days before frozen embryo transfer and (B) control group (n = 56) underwent frozen embryo transfer without intralipid administration. The primary outcome was clinical pregnancy rate (CPR)

Results: Basic and clinical characteristics of the ICSI cycles were matched between groups. In terms of CPR, it was significantly higher in patients received intralipid compared to control group (57.1% versus 37.5%, P value = 0.04). Regarding ongoing pregnancy and miscarriage rates, it was similar in both groups. Univariate analysis revealed that intralipid administration, increased endometrial thickness and lower number of previous failed ICSI were significantly associated with improved clinical pregnancy rates (CPRs). In the multivariate analysis, intralipid administration remained a significant predictor for clinical pregnancy rates.

Conclusion: Intralipid infusion is safe and effective therapy in women with RIF that significantly improved the rates of clinical pregnancy.

Keywords: Intralipid, ICSI, Repeated implantation failure.

INTRODUCTION

After three failed transfers of high-quality embryos, RIF is taken into consideration. In 45% of infertile couples, RIF is still unidentified. RIF could have several factors. Once more, the embryo or endometrium play a major role in the genesis of RIF. The search for uterine deformities, genetic abnormalities, and autoimmune, viral, or endocrine illnesses are all part of a typical workup [(1)].

Women with RIF may have immunological abnormalities, including elevated numbers of peripheral blood natural killer (pNK) cells, deficits in regulatory T cells, and elevated proinflammatory cytokines [(2)]. During the implantation period, uterine natural killer cells (uNK) can make up as much as 70% of the cells in the endometrium. They release several cytokines, such as IL-12, IL-15, Fn-14, IL-18, LIF, and TWEAK, which alter the uterine environment. In women with RIF, an increase of uNK cells and an unbalanced expression of IL-12/Fn-14 and IL-18/TWEAK have been demonstrated to be indicators of immunological profile dysregulation [(3)].

Intralipid is a fat emulsion administered intravenously that contains phospholipids, glycerin, and soybean oil. The primary constituents comprise polyunsaturated fatty acids, such as oleic, palmitic, stearic, linoleic, and α-linolenic acid [(4)]. It is unknown how intralipids directly affect fertility, although it appears to be dependent on the suppression of NK cell cytotoxicity, which may be accomplished by short fatty acids activating PPARγ receptors. The use of intralipid as an immunomodulatory treatment was suggested [(5)].

It has been demonstrated that a single intralipid injection reduces the cytotoxicity of NK cells [(6)]. According to **Dakhly *et al.*** [(7)], RIF is linked to increased NK cell counts and activity as well as boosted production of pro- or anti-inflammatory IL-10 or TNF-α and IFN-γ cytokines in CD4+ T cells. It is believed that pro-inflammatory cytokines activate the activatory receptors on NK cells, increasing their cytotoxicity.

After examining the safety and effectiveness of intralipid therapy in women with unexplained RIF, **Plaçais *et al.*** [(8)] came to the conclusion that intralipid therapy could be a safe and effective way to have a live birth [(8)].

The current study investigated whether intralipid administration could improve the pregnancy outcomes of ICSI cycles in cases with unexplained RIF.

PATIENTS AND METHODS

A randomized controlled trial was conducted at a specialized fertility and gynecology center through the period from January 2022 to December 2023. The study comprised 112 frozen embryo transfer cycles.

Inclusion criteria: Women between the ages of 23 and 39 who had history of at least three failed transfers of high-quality embryos (RIF) and currently

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undergoing frozen embryo transfer of at least one high-quality embryo.

Exclusion criteria: Women older than 39 years. Women with uterine anomalies, autoimmune diseases, endometriosis, fresh embryo transfer, poor embryo quality and refusal to participate in the study. Disorders that interfere with the body's natural metabolism of fat, such as pathologic hyperlipidemia, lipoid nephrosis and poor kidney function.

Randomization: One hundred twelve candidates fulfilled the inclusion criteria were randomly allocated into two equal groups. Group allocation was concealed by opaque sealed envelopes using computer generated randomization sheet by MedCalc © version 13. Each envelope contained a corresponding letter denoting the allocated group.
Participants underwent meticulous history taking, thorough clinical examination, hormonal profile (AMH, TSH and prolactin level), semen analysis, comprehensive transvaginal 3D ultrasound examination (to exclude uterine or tubal causes as uterine septum, polyps, adhesions and hydrosalpinx) and lipid profile.
In both groups, controlled ovarian stimulation was implemented according to long GnRH agonist, short agonist, antagonist or progestin primed protocols. Metaphase II oocytes were injected 3 to 4 hours after retrieval. Fresh transfer of embryos was not included in the current study.

Endometrial preparation for frozen embryo transfer: Oral estradiol valerate (Progynova) was commenced on cycle day 1, in a fixed dose regimen (8 mg/day, 2 mg X 4 times daily). After 12 days of estradiol supplementation, endometrial thickness was measured by transvaginal ultrasound. When endometrium reached optimal thickness (> 8 mm), luteal support was achieved with IM progesterone (100 mg/day) and progesterone vaginal pessaries (400 mg twice per day) together. Embryo transfer was conducted 3 or 4 days after progesterone initiation (cleavage stage) and on the sixth day of progesterone initiation (blastocyst stage).

Treatment protocol: Participants allocated in the intralipid treatment group (n = 56) administrated intravenous infusion of intralipid 100 mL of 20% intralipid diluted in 500 mL of normal saline, approximately 7–10 days before frozen embryo transfer. The control group (n = 56) underwent frozen embryo transfer without intralipid administration.

The primary outcome: CPR (one or more gestational sacs of intrauterine location visualized by ultrasound scan at 5–6 weeks gestation).

Secondary outcomes: Ongoing pregnancy, first trimester miscarriage rates, pregnancy complications and drug side effects. Ongoing pregnancy is a clinical pregnancy continuing beyond 12 weeks of gestational age. The number of pregnancies lost before twelve weeks of gestation divided by the number of women who had a positive pregnancy test was used to calculate the first trimester miscarriage rate.

Sample size estimation: Based on a review of past literature, **Singh *et al.*** **(1)** revealed that the biochemical pregnancy rate in the intralipid group was 40.38% versus control that was 16%. The sample size of the current study was calculated with a significant $P < 0.05$, power of study of 80% and by adding 5% as a drop-out rate. At least 112 patients should be recruited with at least 56 in each group.

Ethical approval: Menoufia Faculty of Medicine's Medical Ethics Committee approved this study. After being informed of all the details, each participant provided written consent. Throughout the course of the investigation, the Helsinki Declaration was adhered to.

Statistical analysis

SPSS version 23.0 for windows was used to statistically analyze the collected data. The Shapiro-Wilk test was used to assume normalcy. When the frequency count was less than 5, the Fischer exact test or Pearson's X^2-test were used for heterogeneity testing. Non-parametric quantitative variables were done using the Independent T test and the Mann Whitney test. When comparing continuous variables, the Student T-test was utilized, with a two-sided probability value of $p \leq 0.05$ considered statistically significant. The mean ± SD of the variables was given. The adjusted odds ratio for the pregnancy outcome was calculated using both univariate and multivariate logistic regression analysis.

RESULTS

One hundred twenty-five candidates were assessed for eligibility to participate in the current study. Thirteen patients were excluded (of these, 9 did not meet the inclusion criteria and 4 declined to participate). So, one hundred twelve participants were available for random allocation into two equal groups (56 in intralipid-treated group and 56 in control group). All participants completed the trial and ready for analysis, as shown in CONSORT flow chart **(Figure 1).**

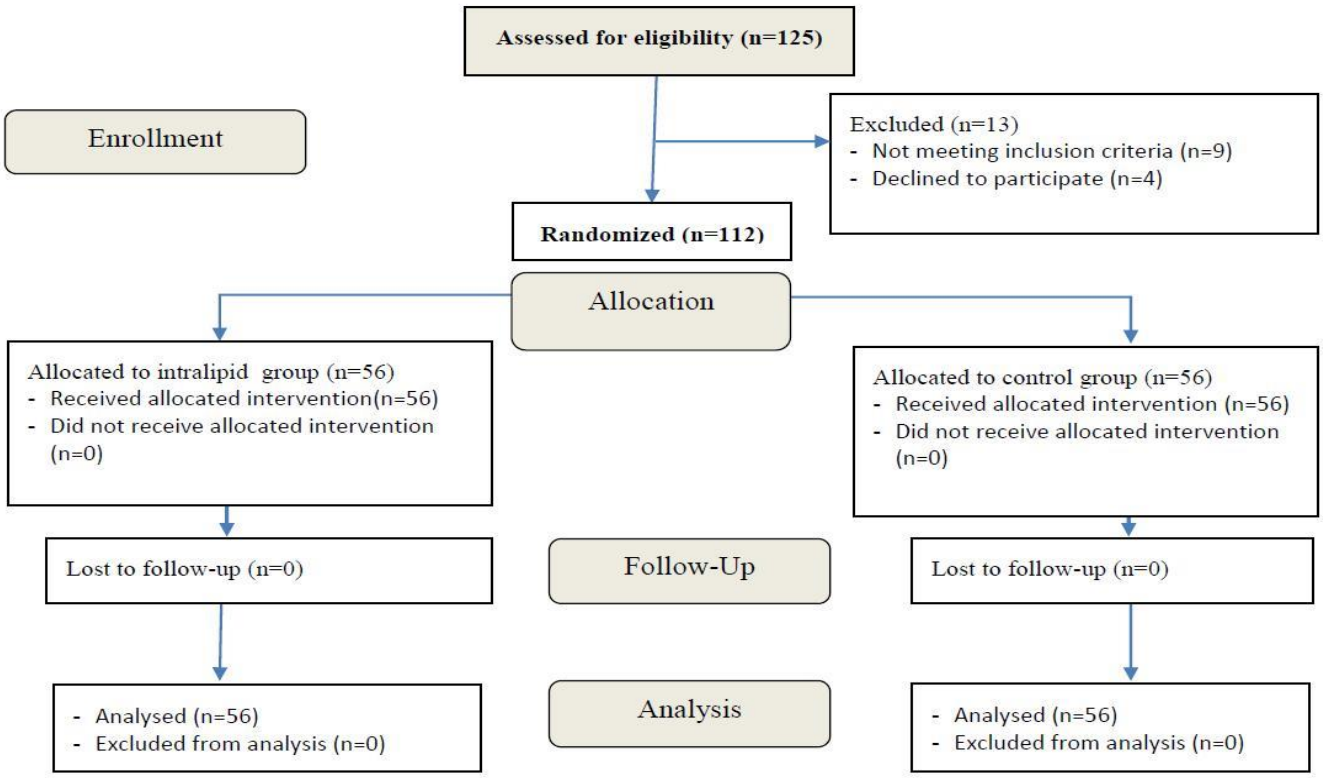

Figure (1): The CONSORT flow chart.

Basic and clinical characteristics of the ICSI cycles were matched between the two groups. No significant differences were noticed between groups as regards age, BMI, duration of subfertility, AMH, number of oocytes retrieved, number of prior IVF cycles, endometrial thickness and number/quality of transferred embryos (Table 1).

Table (1): Basic and clinical characteristics of the ICSI cycles (N=112)

Studied variables	Intralipid group (N=56)	Control group (N=56)	Test of sig U	P value
Age / years	30.1±1.82	30.6±1.99	1.39	0.15
BMI (kg/m^2)	25.9±4.33	24.5±3.64	1.85	0.08
Duration of subfertility (years)	4.31±1.47	4.50±1.57	-0.66	0.51
AMH (ng/ml)	3.81±0.93	3.56±0.61	1.68	0.110
Number of oocytes retrieved	12.43±3.09	11.77±2.69	1.20	0.230
Number of prior IVF cycles	1.57±0.69	1.46±0.57	0.92	0.370
Endometrial thickness (mm)	7.44±1.96	7.24±2.95	0.42	0.340
Number of embryos transferred	1.77±0.79	1.55±0.57	1.69	0.100
Quality of embryos transferred	**N (%)**	**N (%)**	χ^2	
Grade A	50(89.3)	49(87.5)	0.09	0.77
Grade B	6(10.7)	7(12.5)		

On evaluating ICSI outcomes among the studied participants, CPR was significantly higher in patients received intralipid compared to control group (57.1% versus 37.5%, P value =0.04). Regarding ongoing pregnancy and miscarriage rates, it was similar in both groups (Table 2).

Table (2): ICSI outcomes among the studied groups (N=112)

Studied variables	Intralipid Group (N=56)	Control group (N=56)	Test of sig.	P value
Clinical pregnancy	32(57.1%)	21(37.5%)	**χ2** 4.33	**0.04***
First trimester miscarriage	8	6	0.33	0.57
Ongoing pregnancy	24(42.9%)	15(26.8%)	3.19	0.07

*Significant χ^2: Chi square test

There was no significant difference between the studied groups as regards pregnancy complications. No side effects were reported during or after intralipid infusion (flushing, nausea, vomiting, fever or sweating) (Table 3).

Table (3): Pregnancy complications among studied groups

	Intralipid group (n=56)	Control group (n=56)	P-value
Ovarian hyperstimulation	3 (5.4%)	1 (1.8%)	0.31
Pregnancy induced hypertension	2 (3.6%)	1 (1.8%)	0.56
Gestational DM	1 (1.8%)	1 (1.8%)	1.00
Congenital anomalies	1 (1.8%)	0 (0%)	0.32

Univariate and multivariate logistic regression analysis were undertaken to detect any confounding factors that may impact CPRs. In univariate analysis, intralipid administration, increased endometrial thickness and lower number of previous failed ICSI were significantly associated with increased CPRs. However, in multivariate analysis; only intralipid administration and lower number of previous failed ICSI were the only significant predictors for improved CPRs (Table 4).

Table (4): Logistic regression analysis for predictors of clinical pregnancy among the studied groups

Studied variables	OR	P value	95%CI
			Lower – Upper
Univariate analysis			
Age	1.1	0.365	0.89 - 1.34
BMI	1.06	0.290	0.95 - 1.18
Number of previous failed ICSI	0.62	**0.020***	0.24 - 0.89
Endometrial thickness	2.51	**0.040***	1.18 - 8.25
Number of embryos transferred	1.15	0.82	0.87 - 1.86
Intralipid administration	3.88	**0.004****	1.55 - 9.75
Multivariate analysis			
Number of previous failed ICSI	0.60	**0.040***	0.20-0.88
Endometrial thickness	2.14	0.72	0.92-3.59
Intralipid administration	3.12	**0.030***	1.11-8.79

OR: Odds ratio CI: Confidence interval * significant ** highly significant.

DISCUSSION

We investigated the effectiveness and safety of intralipid immunotherapy in women with unexplained RIF in a randomized controlled trial involved 112 frozen embryo transfer cycles. Basic and clinical characteristics of the ICSI cycles were matched with no significant differences between the two studied groups as regards baseline data. Also, **Plaçais *et al.*** [8] examined the effectiveness and safety of intralipid treatment to achieve a clinical pregnancy and live birth, and discovered that there was no discernible difference in age.

On evaluating ICSI outcomes among the current study participants, CPR was significantly superior in patients received intralipid compared to control group (57.1% versus 37.5%, P value =0.04). Regarding ongoing pregnancy and miscarriage rates, it was similar in both groups. Also, **Plaçais *et al.*** [8] found that the intralipid group had considerably higher rates of clinical pregnancy and live births (56% and 55%, respectively) than the non-intralipid group (38% and 33% respectively).

According to our analysis, there were no notable differences between the groups under investigation as regards pregnancy complications and no side effects were reported during or after intralipid infusion. Also, **Plaçais *et al.*** [8] noted no pregnancy complications or side effects that occur during or after the intralipid injection, such as flushing, nausea, vomiting, fever, or sweating.

Univariate and multivariate logistic regression analysis were undertaken to detect any confounding factors that may impact CPRs. In univariate analysis, intralipid administration, increased endometrial thickness and lower number of previous failed ICSI were considered as significant independent factors for increased CPRs. However, in multivariate analysis, only intralipid administration and lower number of previous failed ICSI were the only significant predictors for improved CPRs. Also, **Plaçais *et al.*** [8] used univariate analysis and found that live births in women with RIF were substantially correlated with AMH levels <1 ng/ml and intralipid treatment. Only intralipid medication was shown to be substantially related with live births in multivariate analysis, suggesting that intralipid may be a safe and effective treatment for women with RIF and who also experience recurrent miscarriages.

Our results corroborate those of earlier research examining the possible advantages of intralipids in infertility. **Meng *et al.*** [9] examined the effects of intralipids and immunoglobulins on women who experienced recurrent miscarriages and had peripheral NK cell counts > 20%. In the intralipid group, the success rate of pregnancies was 91%, whereas in the immunoglobulin group, it was 88%.

In agreement with the current observations**, Dakhly *et al.*** [7] employed an intralipid infusion on the day of oocyte retrieval as opposed to a placebo in 296

women with peripheral NK cells >12% and unexplained infertility. The results showed that the intralipid group had a higher percentage of live births (54%) than the placebo group (34%). However, the frequencies of clinical pregnancies and miscarriages did not differ between the two groups.

Besides**, Kumar *et al.* [(10)]** showed that intralipid infusion can improve implantation and LBR in patients with recognized immunological risk factors and/or recurrent miscarriages.

Although the exact mechanism behind the increased live birth rate following intralipid treatment is still unknown, **Roussev *et al.* [(11)]** demonstrated that intralipids affect many immune activities, namely NK cell cytotoxicity. Under intralipid treatment, NK cell activity was considerably reduced, and this effect persisted for four to nine weeks.

Additionally, **Lédée *et al.* [(12)]** documented the in vitro effects of intralipid immunotherapy on T cell activation (reduced) and cytokine production (reduced levels of TNF-α, IL-2, and IL-1β).

On the contrary of our results, according to research including 127 women with unexplained RIF and peripheral NK cells >19%, there was no difference in the number of live births between the group that underwent intralipid infusion and the control group [(13)].

CONCLUSION

Intralipid infusion is safe and effective therapy in women with RIF where significantly improved the rates of clinical pregnancy.

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