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The effect of administration of intravenous intralipid on pregnancy outcomes in women with implantation failure after IVF/ICSI with non-donor oocytes: A randomised controlled trial

Neeta Singh, Amenda Ann Davis, Sunesh Kumar, Alka Kriplani

All India Institute of Medical Sciences, Delhi, India

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ABSTRACT

Objective: Does the administration of intravenous intralipid in women with previous implantation failure at the time of embryo transfer improve pregnancy outcomes in terms of biochemical pregnancy rate, clinical pregnancy rate, ongoing pregnancy rate, and ongoing pregnancy rate?
Study Design: This was a single blinded randomised controlled trial of 105 subjects with previous failed IVF undergoing self donor oocyte IVF/ICSI from January 2017 to May 2018. Randomisation was by computer generated sequence after oocyte pickup. Results were analysed for 102 women, excluding three women due to poor embryo quality. Women in the study arm(n = 52) received 2 doses of 20% intravenous intralipid (Fresenius Kabi), 4 ml diluted in 250 ml normal saline by slow infusion. The first dose was given immediately after oocyte recovery, and the second dose was given on the day of embryo transfer, 1 h prior to the transfer. The control group (n = 50) received normal saline. Flexible ovarian stimulation protocols were used. All the women received routine luteal phase support with micronised vaginal progesterone.
Results: 102 women underwent analysis, 52 in the study group and 50 in control group. There was no significant difference in the baseline characteristics. There was a significant difference in the biochemical pregnancy rate in the intralipid group (40.38%) versus control (16%) [(p = 0.006), RR = 2.5 (1.23–5.16 CI)], clinical pregnancy rate [(34.62% vs 14%), p = 0.006, RR = 2.5(1.13–5.40 CI)], implantation rate [(16.6% vs 6.6%), p = 0.012, RR = 2.5(1.18 to 5.41 CI)], and take home baby rate [28.8% vs 10%, p = 0.024, RR = 2.8(1.1–7.3)]. The adjusted odds ratio for clinical pregnancy in women who received intralipid vs placebo was 3.1 (1.02–9.70 95% CI), p = 0.046. No adverse effects of intralipid were observed.
Conclusion: This study shows a statistically significant increase in implantation rate and live birth rate in women who receive intravenous intralipid with prior implantation failure after IVF/ICSI. These findings concur with other studies; however, literature is limited. The effect of intralipid on the immunological abnormalities in women who experience recurrent implantation failure needs to be investigated further.

Introduction

Assisted reproductive technology has seen advances like no other scientific field since its conception in 1978. One of the mysteries that we still fail to understand is the mechanism and treatment of implantation failure. According to European Society of Human Reproduction and Embryology, 2001, after IVF, the clinical pregnancy rate per aspiration and per transfer was 25.1 and 29.0%, respectively and for ICSI, the corresponding rates were around 26.2 and 28.3% [1]. While these rates have increased, the maximum implantation rate achieved by most ART centres still ranges from only 40 to 60% [2]. An estimated 10% of couples undergoing IVF/ICSI repeatedly experience failure of implantation [3].

Immune dysfunction in the endometrial milieu has been proposed as one of the mechanisms that prevent implantation. Peripheral and uterine natural killers cells have been described as potential culprits in implantation failure and recurrent miscarriages, with increased pre-conceptional activity in women with RPL [4] and demonstrably sustained markers of cytotoxic activity in both peripheral [5] and uterine NK cells [6]. Therapeutic immunomodulatory agents whose effect on their activity have been studied include aspirin [7], progesterone, low molecular weight heparin [8], intravenous immunoglobulin [9], and intralipid, but we still lack sound evidence on any of their efficacy.

Intralipid 20% (a 20% intravenous fat emulsion) is a sterile, non-pyrogenic fat emulsion prepared for IV administration as a source

E-mail address: davis.amenda@gmail.com (A.A. Davis).

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of calories and essential fatty acids. It is made up of 20% soybean oil, 1.2% egg yolk phospholipids, 2.25% glycerin, and water for injection. It is generally indicated for use in total parenteral nutrition to for patients requiring parenteral nutrition for extended periods of time. The hypotheses regarding its role in immune modulation include reduced mitochondrial dependent platelet aggregation [10], reduced hepatic ApoM secretion and enhanced insulin sensitivity [11], altered phospholipid membrane composition of platelets and subsequently reduced aggregation [12], decreased IL-2 production, TNF-α, and IL-1β [13], and long lasting suppression of natural killer cell levels and activity [14].

We conducted a single blinded randomised controlled trial with the hypothesis that the administration of intravenous intralipid to women with previous implantation failure after non-donor oocyte IVF/ICSI at the time of oocyte retrieval and embryo transfer would lead to better IVF outcomes measured in terms of biochemical pregnancy rate, clinical and ongoing pregnancy, implantation rate, live birth rate, and take home baby rate.

Materials and methods

Our study was a prospective single blinded randomised placebo controlled trial conducted at the Assisted Reproductive Centre, Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi, India from January 2017 to January 2018 and included 105 women with primary infertility in the age group of 20–40 years undergoing non donor oocyte IVF/ICSI with at least one previous implantation failure. Women with recurrent miscarriages were not included as they would constitute secondary infertility. Prior to enrolment, the women were assessed to have normal hormonal profile of FSH, LH, TSH, PrL, and AMH, normal uterine cavity assessed by hysteroscopy, body mass index in the range of 20–32 kg/m2, and morphologically normal oocytes and embryos. We excluded women with obvious anatomical causes for IVF failure like fibroids or polyps as assessed by hysteroscopy, age more than 40 years, women undergoing donor oocyte IVF, poor quality embryos, and diagnosed medical comorbidities or thrombophilias. Women with any known allergic predisposition to eggs, lecithin, or soy products were excluded as well. Written and informed consent was taken from all the couples in the study. Institute ethical clearance was taken before undertaking the study. Randomisation was done using 1:1 computer generated sequences immediately after oocyte retrieval, to either intralipid or placebo. Participants in the study were unaware of the nature of the intervention they were to receive and allocation concealment was done using brown paper bags. We did not perform sample size calculation due to lack of previous studies.

Pre IVF assessment included a detailed history and examination including pelvic examination. Baseline ovarian reserve was assessed using day 2 or day 3 serum FSH, LH, AMH and ultrasound assessment of antral follicular count and ovarian volume. Secretory phase endometrial thickness and pattern were assessed by transvaginal ultrasound. Women with history of tuberculosis were treated prior to enrolment for IVF. Semen analysis of male partner was done to rule out severe male factor infertility and any infections detected on culture were treated. We excluded couples with severe male partner infertility requiring surgical sperm retrieval, but donor semen cycles were included.

Controlled ovarian stimulation was done according to flexible protocols based on the women's age, BMI, and previous cycle response. Agonist protocol involved down regulation with leuprolide acetate (0.5 mg) from mid-luteal phase followed by stimulation with recombinant FSH (Gonal F, Merck Serono, Mumbai). In the antagonist protocol, rFSH was started from 2nd or 3rd day, and when a single follicle of >/−14 mm was seen on ultrasound, GnRH antagonist cetrotide (Cetrorelix, Merck Serono, Mumbai) was given. Oocyte trigger was done using recombinant hCG 250 ug subcutaneous (Ovitrelle; Merck Serono, Mumbai, India) when at least two leading follicles of ≥18 mm were obtained and retrieval done 24 to 36 h after trigger when at-least 3 follicles >18 mm in diameter were visible. Oocytes were rinsed, graded and placed in Vitrolife G-MOPSTM media (Vitrolife Sweden AB, Göteborg, Sweden), and inseminated or injected 2 to 4 h later with semen. Fertilisation was checked 18 h after insemination, and embryos transferred on day 2, 3 or 5 depending on grading and number available.

The study group (n = 53) was administered intravenous infusion of 4 ml Intralipid 20% (Fresenius Kabi) in 250 ml normal saline, the first dose on the day of oocyte retrieval (after retrieval). The second dose (same as the first dose) was given on the day of embryo transfer, one hour prior to transfer, to (n = 52) women. The control group received normal saline infusion instead of intralipid. The first dose was given to 52 women and the second dose to 50 women. We excluded 1 woman in the study group and 2 in the control group due to poor embryo quality or failed fertilisation. The intralipid was given over one hour with monitoring for any allergic reaction. All the women received luteal phase support in the form of parenteral or vaginal micronised progesterone.

The primary outcomes measured were biochemical pregnancy rate (defined as a B-HCG >/-100 IU 14 days after embryo transfer), and clinical pregnancy rate (ultrasound confirmed intrauterine pregnancy with cardiac activity), live birth rate (any birth event after 24 weeks gestation resulting in the delivery of a live infant), implantation rate (number of gestational sacs implanted per total number of embryos transferred), and take home baby rate(number of healthy babies taken home with no early neonatal complications).

Statistical analysis was done using Stata SE 13.0 (Stata Corps, TX, USA). Normality assumption was done using Shapiro Wilk test. Heterogeneity testing was done using Pearson's Chi square or Fischer's exact test when frequency count was less than 5. For continuous variables, mean and standard deviations were reported and comparison done with Student independent T-test. For comparing outcomes between the two groups we calculated the relative risk, taking a two sided probability value of $p < 0.05$ as statistically significant and a 95% confidence interval. Univariate and multivariate logistic regression analysis was performed to calculate adjusted odds ratio for pregnancy outcome.

Results

A total of 105 women were enrolled and randomised to received intralipid(n = 53) or placebo(n = 52), and 102 (n = 52 and 50 in respective groups) received both the allocated doses and underwent analysis (Fig. 1) the participants were well matched in both groups in terms of age, BMI, mean cycle number, and ovarian reserve. There was no significant difference in the stimulation protocols used in either group (Table 1).

Biochemical pregnancy rate, defined as B-HCG >/-100 IU 14 days after embryo transfer was significantly higher in the Intralipid group than control (40.4% versus 16%, p = 0.006). Clinical pregnancy rate, defined by ultrasonographic features of live intrauterine pregnancy, was significantly higher in the intralipid group as compared to controls (34.6% versus 14%, p = 0.023), and all of these pregnancies were ongoing at 16 weeks gestation and resulted in live births. Implantation rate, calculated by taking total number of embryos transferred in the denominator (144 in the intralipid group and 126 in the study group), was significantly higher in the implantation group. (16.6% vs 6.5%, p = 0.012). For calculating live birth rate, we considered twin gestations as a single entity. There was also a significant difference in the take home baby rate, for which we considered each twin as a separate entity (28.8% vs 10%,

p = 0.024). There was no significant difference in the rate of multiple pregnancies (Table 2).

Univariate and multivariate logistic regression analyses were performed to identify any confounding variables which may have affected pregnancy rates. We considered age, BMI, day of embryo transfer, number of embryos transferred, and history of tuberculosis, mean cycle number, and endometrial thickness as variables for univariate analysis. Variables with $p < 0.25$ were considered for multivariate analysis. The adjusted odds ratio for clinical pregnancy with Intralipid was 3.14 (1.02–9.70 at 95% CI), p = 0.046. The day of embryo transfer (day 5 and 3 versus day 2 transfer), and endometrial thickness more than or equal to 8 mm

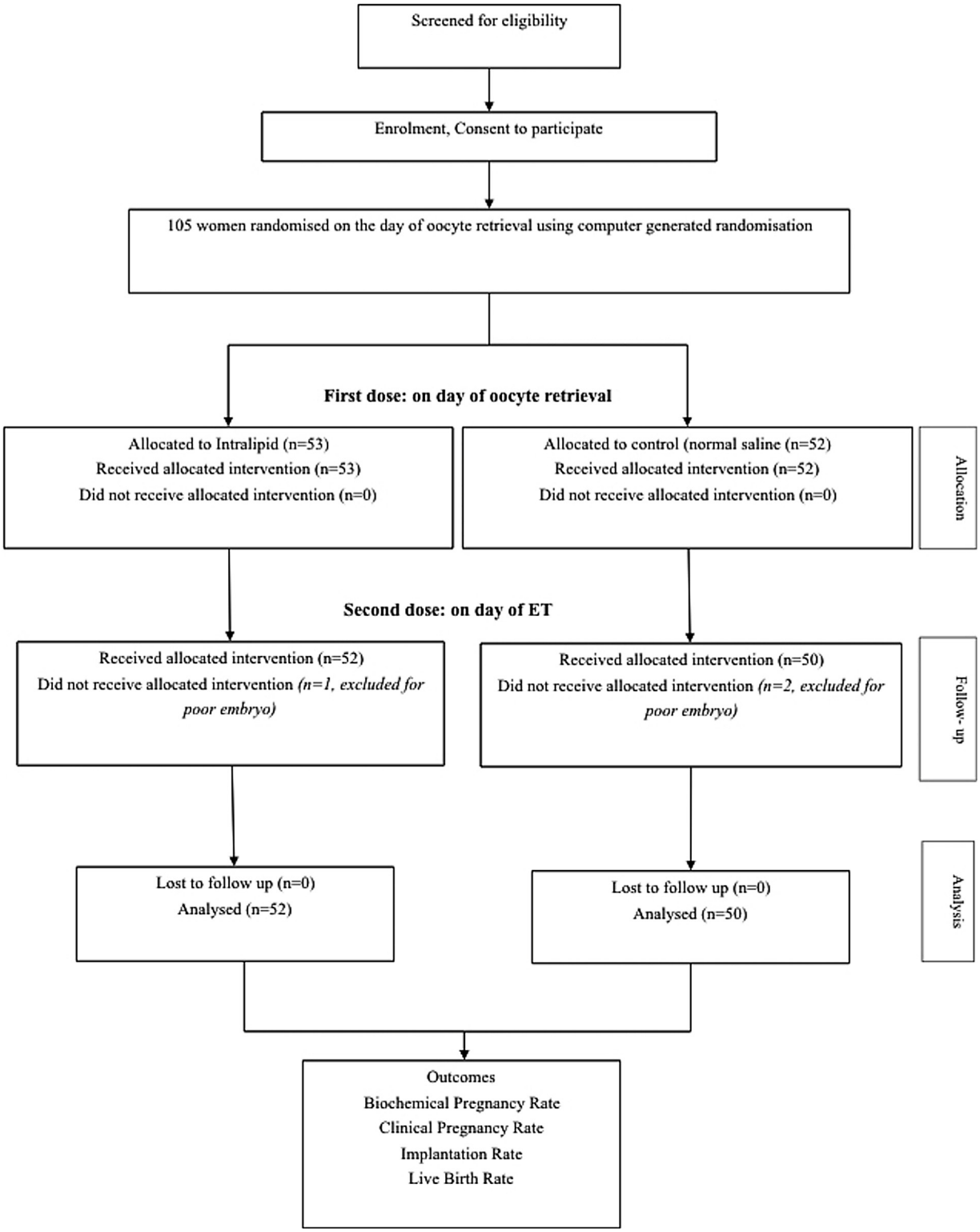

Fig. 1. CONSORT Flow of Participants.

Table 1
Baseline Characteristics.

Participant Characteristics	Study Group (n=52) (%)	Control Group (n=50) (%)	P-value
Age in years (Mean±SD)	31.64 ± 3.96	32.12 ± 3.50	0.52
BMI (kg/sq. m) (Mean±SD)	24.06 ± 3.12	24.61 ± 3.32	0.39
Etiology of Infertility			
Both unexplained	11 (21.15)	7(14)	0.34
Either partner	37 (71.15)	41(82)	0.20
Both partners	4(7.69)	2(4)	0.68
Male Factor Infertility			
None	39(75)	39(78)	0.72
Oligozoospermia	4(7.69)	1(2)	0.36
Azoospermia	9(17.30)	10(20)	0.99
Female Factor Infertility			
Unexplained	19(36.53)	18(36)	0.96
Diminished ovarian reserve(DOR)	5(9.61)	2(4)	0.44
Tubal	13(25)	21(42)	0.07
Endometriosis	1(1.92)	4(8)	0.20
Polycystic ovarian syndrome (PCOS)	6(11.53)	3(6)	0.49
Tubal and PCOS	3(5.76)	2(4)	0.99
Tubal and DOR	4(7.69)	0	0.12
Tubal and Endometriosis	1(1.92)	0	0.99
Mean Cycle Number (Mean ±SD)	2.32 ± 0.58	2.3 ± 0.50	0.85
Mock ET			
Difficult	7(13.46)	4(8)	0.53
Day 2 Hormone Profile (Mean ±SD)	4.51 ±3.31	4.39 ±2.04	0.83
AMH(ng/mL)	5.16 ±2.50	4.05± 3.50	0.07
LH(ng/mL)	6.32 ±2.24	6.14± 4.69	0.80
FSH(ng/mL)			
Ovarian Reserve on USG (Mean ±SD)	8.05± 3.98	7.44± 3.05	0.39
Right Ovary AFC	7.40± 3.33	7.28± 3.16	0.85
Left Ovary AFC	15.46± 6.98	14.72±5.67	0.58
Total AFC	4.95± 2.42	5.46± 2.16	0.26
Right Ovary Volume (mL)	4.49± 1.84	5.11± 2.21	0.13
Left Ovary Volume (mL)			
Procedure done	31(59.61)	33(66)	0.51
IVF (with husband semen)	10(19.23)	7(14)	0.48
IVF donor semen	11(21.15)	10(20)	0.92
ICSI			
Stimulation Protocol	22(42.30)	30(60)	0.07
Agonist	25(48.07)	15(30)	0.06
Antagonist	5(9.61)	5(10)	0.99
Microdose Flare			
Days of stimulation (Mean±SD)	10.84±1.68	11.28±1.66	0.20
Starting dose of FSH (Mean±SD)	299.74±59.81	271.18±78.32	0.11
(IQR)	(275-375)	(212-337)	
Starting dose of HMG(Mean±SD)	94.56± 64.09	170.83± 128.79	0.009
(IQR)	(75-100)	(75-375)	
Total dose of FSH(Mean±SD)	3057.72±796.58	2355.65± 836.93	
IQR	(2409- 3600)	(1800- 2962.5)	0.002
Total dose of HMG(Mean±SD)	674.22± 797.94	1442.16± 1127.52	
IQR	(300-675)	(375-2435)	0.002
Dose of Cetrotide (Mean±SD)	1.42±0.49	1.38±0.51	0.80
E2 on day of trigger (Mean±SD)	3401.01± 1417.03	3640.68± 1435.78	0.56
(IQR)	(2170-4868)	(2442± 4800)	
P4 on day of trigger (Mean±SD)	1.3±0.8	1.5±1.4	0.33
No. of Oocytes retrieved (Mean ±SD)	5.96±3.45	4.62±1.99	0.09
Endometrial Thickness (Mean in mm±SD)	8.05±1.04	8.48±1.79	0.14
Endometrial Pattern	36(69.23)	42(84)	0.08

Type A Pattern (Trilaminar) Type C (Diffuse)	16(30.76)	8(16)	0.08
Day of ET	15(28.84)	11(22)	0.08
Day 2	14(26.92)	24(48)	0.03
Day 3	23(44.23)	15(30)	0.14
Day 5			
Number of Embryos Transferred			
Mean ± SD	2.09±1.1	2.48±1.07	0.19
Median	2	2	
IQR	1.5-3	2-3	

Table 2
IVF Outcomes.

	Study Group (n = 52) (%)	Control Group (n = 50) (%)	P-value	RR(95% CI)	NNT
Biochemical Pregnancy	21(40.38)	8(16)	0.006	2.52(1.23–5.16)	4.1
Clinical Pregnancy Rate	18(34.62)	7(14)	0.023	2.5(1.13–5.40)	4.9
Implantation Rate	16.60	6.60	0.012	2.5(1.18 – 5.41)	9.9
Ongoing Pregnancy Rate (16 weeks)	18(34.62)	7(14)	0.023	2.5(1.13–5.40)	4.9
Live Birth Rate	18(34.62)	7(14)	0.023	2.5(1.13–5.40)	4.9
Single pregnancies	12(23.08)	6(12)	0.149	2.1(0.87–5.20)	7.2
Twin pregnancies	6(11.54)	1(2)	0.078	6.6(0.83–52.47)	7.8
Take home baby rate (%)	28.8	10	0.024	2.8(1.1–7.3)	5.3

were positively associated with pregnancy. The odds ratio for successful pregnancy was higher in women with only one previous failed IVF compared to those with 2 or more attempts (Table 3). Adverse pregnancy outcomes in either group were not statistically or clinically significant (Table 4). No adverse effects of the intervention were observed.

Discussion

This study demonstrates a significant effect of intravenous intralipid on pregnancy outcomes in IVF. There was a higher biochemical pregnancy rate, clinical pregnancy rate, implantation rate, and take home baby rate in the intralipid arm which was

Table 3
Multivariate Logistic Regression Analysis.

Variable	Univariate Analysis Odds Ratio (95% CI)	P-value	Multivariate Analysis Adjusted Odds Ratio (95% CI)	P-value
Intralipid administration				
Intralipid administered	2.98 (1.11-8.01)	0.02	3.14 (1.02-9.70)	0.046
Control	1 (Reference)	1		
Age				
20–24	1(Reference)	1		
25–29	NS	0.99		
>/=30	NS	0.99		
BMI				
19–24 kg/m2	0.92 (0.15-5.56)	0.92		
25–30 kg/m2	1 (Reference)	1		
>30 kg/m2	0.43 (0.21-0.91)	0.03		
Endometrial thickness				
<8mm	1 (Reference)	1		
8–12 mm	3.51 (1.18-10.40)	0.02	3.17 (0.97-10.32)	0.06
>12 mm	7.6 (0.41-141.54)	0.36	21.84 (0.91-518.83)	0.06
Day of ET				
Day 2	1 (Reference)	1		
Day 3	1.04 (0.26-4.12)	0.95		
Day 5	3.66 (1.04-12.84)	0.04	3.75 (0.96-14.49)	0.06
History of TB				
Yes	0.55 (0.11-2.69)	0.46		
No	1 (Reference)	1		
Number of previous failed IVF				
1	5.5 (1.2-25.20)	0.03	5.91 (1.11-31.42)	0.037
2 or more	1 (Reference)	1		
Number of embryos transferred	1.01 (0.63-1.63)	0.94		

In univariate analysis, the reference variable within each sub-category has been arbitrarily designated and given the odds ratio of 1, with the odds ratio for the other variables calculated in comparison to the reference. Variables with $p < 0.25$ were considered for multivariate analysis.

Table 4
Complications in Pregnancy.

	Study Group	Control Group	P-value	Details
Pregnancy Loss	1	0	0.5	One patient with twin pregnancy had intrauterine fetal demise. Macerated 988 gm baby was delivered (CS) along with live twin.
Ovarian hyperstimulation	1	1	0.9	One patient had mild OHSS in the study group. One patient had moderate OHSS in the control group which required hospital admission.
Pregnancy induced hypertension	3	0	0.24	Two patients had mild gestational hypertension. One patient required LSCS for severe pre-eclampsia
Gestational diabetes	2	0	0.50	One patient with GDM required insulin from early pregnancy.
Preterm Labor	1	1	0.9	One patient had preterm prelabor rupture of membranes and underwent emergency LSCS in the study group. In the control group, one patient went into preterm labor and delivered 2 live babies who later expired of sepsis.
Congenital Anomalies	1	0	0.5	One patient in the study group had congenital diaphragmatic hernia in the fetus.
Neonatal complications	0	1	0.49	Neonatal sepsis occurred in one patient who had preterm labor due to E.coli. Both the twin babies expired.

statistically significant. This was appreciated even after adjusting for confounding variables.

This was a single blinded study with only the participants were masked to the intervention. Our inclusion criteria did not meet the current definitions of "recurrent implantation failure" and we found the beneficial effect of intralipid more appreciable in women with only one previous failed IVF. Other factors which we found to influence the outcome were the endometrial thickness and a later day of embryo transfer. We did not perform any test to identify women with immune dysfunction such as measuring the natural killer cell activity, and hence cannot comment upon whether this positive effect can be extrapolated to all women or only those with evidence of immune dysregulation.

The findings of the present study generally concur with previous studies evaluating the effect of intralipid in implantation failure during IVF. Acacio et al. (2008) reported in a non randomised study a pregnancy rate of 40% in women with a previous history of pre or post implantation pregnancy failure [15]. Ndukwe et al. (2011) presented the findings of a non-randomised study in a scientific meeting in which they found a 46% clinical pregnancy rate in women with recurrent implantation failure with elevated cytokine TH1 response [16]. Coulam et al. (2012) similarly had a 52% pregnancy rate in women with RIF or recurrent abortions [17]. El Khayat et al (2015) conducted a randomised trial in 203 women with RIF following ICSI and found a statistically significant increase in pregnancy outcomes in women who received intralipid [18]. Dakhly et al. (2016) in a randomised study in women with recurrent spontaneous abortions found a significantly higher ongoing pregnancy rate and live birth rate but not biochemical pregnancy rate, perhaps because they included women with secondary infertility were experiencing clinical pregnancy losses [19]. Table 5 shows a comparison of these two trials with the present study, demonstrating a similar live birth rate in all the three studies.

Benschop et al. (2012) had outlined the protocol for a Cochrane review of immune therapy, including intralipid, for implantation failure [20]. Preliminary results of this Cochrane review were presented at the 34th Annual Meeting of the ESHRE in 2018, which suggested that intralipid probably improves live birth rate compared to no treatment (RR 2.13, 95% CI 1.35–3.36) [21]. While evidence of immunomodulation at the molecular level builds, we still do not know the exact mechanism by which intralipid acts. What we can conclude is that intralipid may alter the uterine environment in favour of the TH2 cytokines, and alter the uterine natural killer cell phenotype to one that favours pregnancy. Considering the available evidence, as well as the biological plausibility of intralipid, it may be beneficial to offer intralipid therapy to couples who have previously experience implantation failure. The best way forward may be to compare immunological testing in women who successfully have live birth after intralipid versus women who do not. Until the exact intricacies of endometrial receptivity are fully elucidated, the need for larger, better powered randomised controlled studies cannot be over-emphasised.

Table 5
A comparison of 3 Randomized Trials of Intralipid.

Author Sample Size	El Khayat [18] 203	Dakhly [19] 296	Present Study 105
Inclusion Criteria	Repeated implantation failure defined as failure to achieve pregnancy after two to six ICSI cycles with the transfer of more than ten high grade embryos.	Women with unexplained secondary infertility, Recurrent, and elevated levels of natural killer cells (>12%)	Women with primary infertility, at least one previous implantation failure
Dose of Intralipid	Intralipid, 4 ml 20% between day four and nine of ovarian stimulation during ICSI cycles & another dose when got pregnant within the 1 st week of positive pregnancy test	2mL diluted at 20% in 250 ml saline on the day of oocyte retrieval. Repeated 1 weeks after positive pregnancy test and then 2 weekly	4 ml Intralipid 20% (Fresenius Kabi) in 250 ml normal saline, the first dose on the day of oocyte retrieval (after retrieval). The second dose (same as the first dose) was given on the day of embryo transfer, one hour prior to transfer.
Clinical Pregnancy Rate (Study Group)	35%	58.3%	34.6 %
Live Birth Rate (Study Group)	33%	37%	34.6 %
Odds ratio for live birth	1.6 (1.28 - 2.13)	2.082 (1.251–3.465)	2.5 (1.13-5.40)

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