A Therapeutic Protocol in Previous Failed ART Patients with High Total NK Cells: Randomized Controlled Trial

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Abstract
Background: Failed assisted reproductive technology (ART) is a challenging fact. Therefore, different immunomodulatory agents are being investigated. This study aimed to assess a novel therapeutic protocol consisting of progesterone (IM and suppository), low-dose aspirin, folic acid, lipid emulsion, prednisolone, and low molecular weight heparin (LMWH).
Methods: One hundred patients were randomized into control and interventional groups (50 females for each). The control group underwent the standard treatment consisting of progesterone, folic acid, and low-dose aspirin. In addition to the standard treatment, the interventional arm received lipid emulsion, prednisolone, and low molecular weight heparin (LMWH).
Results: The interventional group demonstrated significantly higher chemical (54.0% vs. 40%) and clinical (40.0% vs. 30%) pregnancy rates than the control group but without statistical significance (P = 0.161 and 0.295). Furthermore, the interventional group showed higher ongoing pregnancy (85%) than the control arm (60%), with a borderline significance (P = 0.094)
Conclusion: Intravenous lipids could improve pregnancy outcomes in patients with high Natural Killer (NK) cells and failed ART. Further studies with larger sample sizes are recommended to confirm the value of intralipids and adopt their use in clinical practice.

Keywords: NK cells, Intravenous lipid, In vitro fertilization

Introduction

Failed assisted reproductive technology (ART) has been challenging for reproductive scientists and embryologists. Recently, studies have suggested that immune factors play an essential role in its pathogenesis [1].

The main causes for failed ART include poor sperm, oocyte, or embryo quality, uterine or tubal anomalies, infection, thrombophilia conditions, or immunological dysfunction. An interaction of immunological and hormonal factors is required to establish optimal endometrial receptivity for effective blastocyst implantation and subsequent deep placentation. It is hypothesized that immunological dysregulation contributes to improper implantation and deep placentation, causing pregnancy challenges such as recurrent loss [2].

Over the past two decades, Natural Killer (NK) cells have been accused of unsatisfactory reproductive outcomes, such as recurrent implantation failure (RIF) or recurrent miscarriage (RM). Despite contradictory evidence, numerous reproductive clinics have offered immunotherapy to various patient groups based on aberrant NK cell numbers or activity [3].

In a normal pregnancy, uterine NK cells provide sufficient cytokine support and local immunomodulation via direct or indirect progesterone actions, whereas peripheral NK cells downregulate their activity. In recurrent miscarriage, creating a peripheral Th1 cytokine milieu may result in NK cell activation and proliferation, triggering cytotoxic NK cells migration into the uterus and causing miscarriage [4].

Several immunomodulatory medications affecting NK cells have been studied, including aspirin [5], low molecular weight heparin (LMWH) and progesterone [6], intravenous immunoglobulin [7], and intralipid. However, solid evidence of their effectiveness is still lacking.

This study aimed to assess a novel therapeutic protocol consisting of progesterone (IM and suppository), low-dose aspirin, folic acid, lipid emulsion, prednisolone, and low molecular weight heparin (LMWH).

Patients and methods

This prospective randomized controlled trial (RCT) was conducted in the Gynaecology and Obstetrics Department, Benha University Hospital, and Hawaa Fertility Center on 100 cases with high NK cells and failed ART cycles. Patients were randomized into control and interventional groups (50 females for each). The control group underwent the standard treatment consisting of progesterone, folic acid, and low-dose aspirin. In addition to the standard treatment, the interventional arm received lipid emulsion (in the cycle before embryo transfer), prednisolone (with the onset of lipid emulsion), and LMWH (on the day of embryo transfer).

This research was conducted following Helsinki's declaration, and the study was approved by the institutional review board (IRB), Faculty of Medicine, Benha University (Approval number: 12-7-2023). Each participant consented after being fully informed about the study purpose and procedure. The study protocol was registered at ClinicalTrials.gov (Identifier: NCT03792997).

Inclusion and exclusion criteria

Inclusion criteria were adult females with unexplained infertility and high peripheral NK cells > 10%. Exclusion criteria included females with infertility due to uterine or male factors and those with a poor response (less than 3 MII oocytes).

Randomization and blinding

Randomization was done using computer-generated random numbers. The allocation sequence was concealed from the study investigators in sealed envelopes to ensure the unbiased allocation of participants to the study arms. Envelopes containing the allocation data were selected sequentially by the patient in the presence of the study nurse. The study was single-blinded, with all investigators being blinded to patients' allocation.

Outcome measures

The primary outcome of the study was chemical pregnancy within 14 days of embryo transfer. Chemical pregnancy was assessed using serum levels of hCG, a hormone produced by the developing embryo after implantation. The secondary outcome was clinical pregnancy within six weeks of gestation. It was assessed by visualizing the gestational sac within the uterus using an ultrasound examination.

Sample size calculation

G*Power software version 3.1.9.2 was used for sample size calculation. Based on a previous study by Singh N et al., the pregnancy rate was 40.38% in the intravenous intralipid and 16% in the control arm [8]. The total sample calculated was 100 (50 in each arm). Alpha and power were kept at 0.05 and 0.8, respectively.

Statistical methods

SPSS version 25 was utilized for statistical analysis (IBM Armonk, New York, United States). Using the Kolmogorov–Smirnov test and direct data visualization techniques, the normality was evaluated. Means and standard deviations or medians and ranges were used to summarize quantitative data based on the results of the normality testing. Frequencies and percentages were used to summarize the categorical data. The unpaired t-test and Mann-Whitney U test compared normally and non-normally distributed quantitative variables, respectively, between the study groups. The Chi-square test was used to compare categorical data. Each statistical test was two-tailed. P values were considered significant if less than 0.05.

Results

One hundred and thirteen females were assessed for eligibility. One hundred females fulfilled the eligibility criteria. They were randomized into two equal groups. The CONSORT flow diagram is shown in Figure 1.

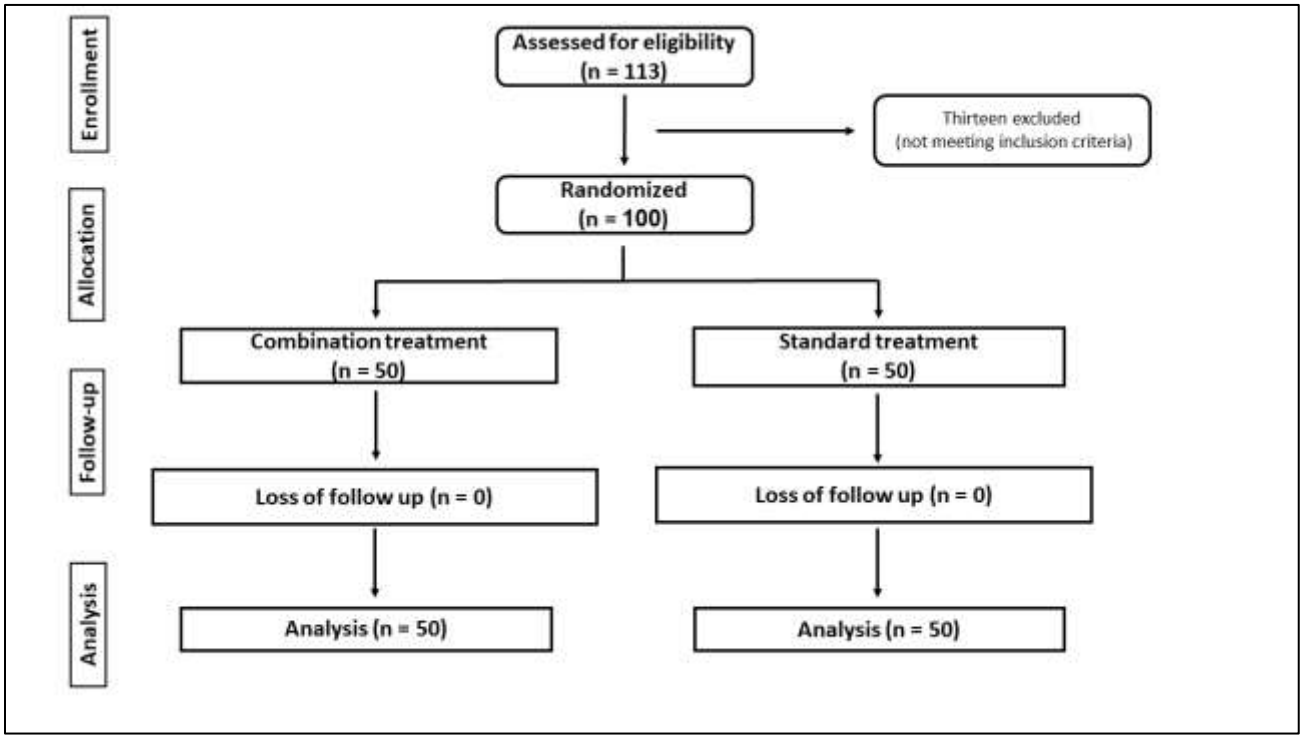

Figure : CONSORT flow diagram of the study participants

As shown in **Table 1**, the studied groups were comparable regarding all general characteristics, including age (P = 0.179), husband age (P = 0.2), marriage duration (P = 0.167), NK cells (P = 0.826), FSH (P = 0.821), and endometrial thickness (P = 0.394).

Table (1) General characteristics of the studied groups

		Intervention (n = 50)	**Control (n = 50)**	**P-value**
Age (years)	Mean ±SD	28 ±5	30 ±4	0.179
Husband age (years)	Mean ±SD	34 ±5	32 ±5	0.2
Marriage duration (years)	Median (range)	5 (2 - 14)	6 (3 - 13)	0.167
NK cells (%)	Mean ±SD	15.2 ±2.8	15.1 ±2.6	0.826
FSH (days)	Mean ±SD	10 ±1	10 ±1	0.821
Endometrial thickness (mm)	Mean ±SD	9 ±2	8 ±2	0.394

NK: Natural killer; FSH: Follicle-stimulating hormone

Regarding the outcomes, the interventional group demonstrated significantly higher chemical (54.0% vs. 40%) and clinical (40.0% vs. 30%) pregnancy rates than the control group but without statistical significance (P = 0.161 and 0.295) (Table 2). Furthermore, the interventional group showed higher ongoing pregnancy (85%) than the control arm (60%), with a borderline significance (P = 0.094) (Table 2 & Figure 1).

Table (2) Pregnancy outcome in the studied groups

		Intervention (n = 50)	Control (n = 50)	P-value
Chemical pregnancy	n (%)	27 (54.0)	20 (40.0)	0.161
Clinical pregnancy	n (%)	20 (40.0)	15 (30.0)	0.295
Ongoing pregnancy *	n (%)	17 (85.0)	9 (60.0)	0.094

* Percentages were calculated based on those with a positive clinical pregnancy

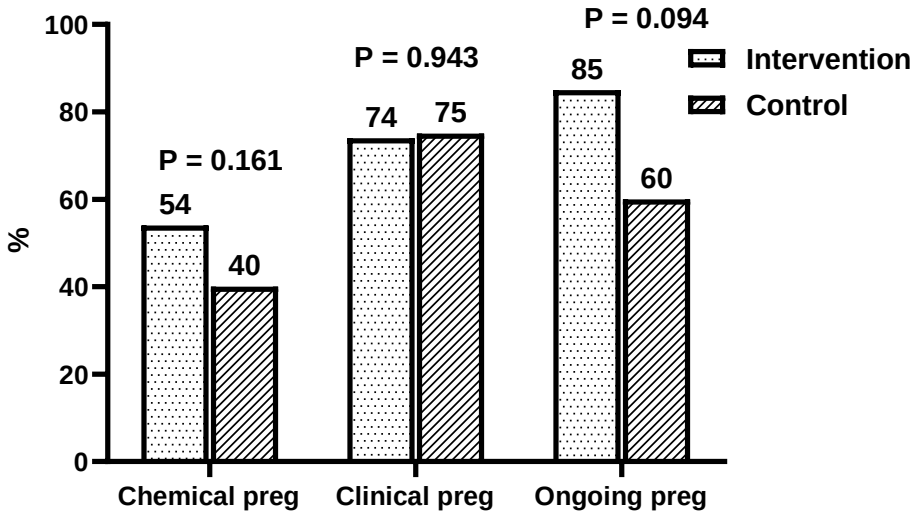

Figure 3: Pregnancy outcome in the studied groups

Discussion

Recurrent implantation failure (RIF) is an unfortunate reality. In many ART clinics, the highest successful implantation rate ranges from 40% to 60% [9]. Different therapeutic immunomodulatory agents are being investigated to overcome this problem. Although immunomodulation has developed as an alternative therapy for in vitro fertilization (IVF) patients in recent years, the specific mechanism through which intravenous lipid exerts its effects remains unknown. The current study assessed whether a combined therapy of lipid emulsion, LMWH, and prednisolone would result in better chemical, clinical, and ongoing pregnancy rates than the standard therapy alone.

In the current study, randomization managed to achieve comparable groups regarding all potential confounders, including female age, husband age, marriage duration, NK cells, follicle-stimulating hormone (FSH), and endometrial thickness, ensuring the internal validity of the study.

The current study indicated that chemical, clinical, and ongoing pregnancies were better in the interventional arm than in the control arm but without statistical significance. Only the ongoing pregnancy achieved a borderline significance. El Khayat et al., in a randomized trial, demonstrated significantly higher pregnancy rates in patients receiving intralipid [10]. Another trial by Dakhly et al. on women with elevated NK cells and recurrent spontaneous abortions emphasized that females receiving intralipid have significantly higher ongoing pregnancy rates, not biochemical ones [11].

In a more recent trial, Singh et al. assessed the effect of intravenous intralipid on pregnancy outcomes in women with implantation failure after failed ART. They demonstrated that intravenous intralipid improves all pregnancy rates; chemical, clinical, and ongoing [8].

Additionally, a recent case-control study reported significantly higher chemical and clinical pregnancy rates in the intravenous lipid group than in the control group [12]. Moreover, in vitro research demonstrated that intralipids inhibit NK cytotoxicity [13]. In a related study, Ledee et al. evaluated intravenous lipid therapy in females with unexplained RIF undergoing ART and found an improvement in the live birth rate [14].

In contrast, other researchers indicated that there is no benefit of intralipid treatment. Early results of a case-control study revealed no differences in pregnancy outcomes between the intralipid and the control groups [15]. Additionally, a recent meta-analysis indicated insufficient evidence to warrant the use of intralipid during embryo transfer in women with a history of RIF [16].

Despite the statistical insignificance of pregnancy rates in the current study between the studied groups, the clinical differences are evident in favor of intralipid therapy and should not be disregarded. The non-statistical significance could be attributed to the small sample size. The sample size was calculated based on a study that reported a wider effect size than the one found in this study, and a wider effect size leads to a smaller sample.

The main strength of the current study is being an RCT. RCTs ensure unbiased allocation of treatments and minimize potential confounding factors, enhancing the internal validity of the study and rendering it an important contribution to the field of reproductive medicine. Another strength is that all participants had high NK cells, enhancing the generalizability of the results to similar patients. A possible limitation is the relatively small sample size.

Conclusion

Intravenous lipids could improve pregnancy outcomes in patients with high NK cells and failed ART. Further studies with larger sample sizes are recommended to confirm the value of intralipids and adopt their use in clinical practice.

Acknowledgments

None.

Authors' contributions

MH, AE, and AHA designed and supervised the study. AS, MME, ASR conducted the study and analyzed the data. MAR analyzed the data. All authors wrote and approved the manuscript.

Funding

This research received no funding support.

Data availability

The data supporting the findings are available upon reasonable request.

Declarations

Ethics approval

The study was approved by the institutional review board (IRB), Faculty of Medicine, Benha University (approval number: 006).

Consent for publication

Not applicable.

Competing interests

None to be declared.

References

1- Makrigiannakis, A., Petsas, G., Toth, B., Relakis, K., & Jeschke, U. (2011). Recent advances in understanding immunology of reproductive failure. J. Reprod. Immunol., 90(1), 96–104.

2- Brosens, I., Pijnenborg, R., Vercruysse, L., & Romero, R. (2011). The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. Am. J. Obstet. Gynecol., 204(3), 193–201.

3- Moffett, A., & Shreeve, N. (2015). First do no harm: uterine natural killer (NK) cells in assisted reproduction Hum. Reprod., 30(7), 1519–1525.

4- Dosiou, C., & Giudice, L. C. (2004). Natural Killer Cells in Pregnancy and Recurrent Pregnancy Loss: Endocrine and Immunologic Perspectives. Endocr. Rev., 26(1), 44–62.

5- Gelbaya, T., Kyrgiou, M., Li, T., Stern, C., & Nardo, L. (2007). Low-dose aspirin for in vitro fertilization: a systematic review and meta-analysis. Hum. Reprod. Update., 13(4), 357–364.

6- Berker, B., Taşkın, S., Kahraman, K., Taşkın, E. A., Atabekoğlu, C., & Sönmezer, M. (2011). The role of low-molecular-weight heparin in recurrent implantation failure: a prospective, quasi-randomized, controlled study. Fertil. Steril., 95(8), 2499–2502.

7- Li, J., Chen, Y., Liu, C., Hu, Y., & Li, L. (2013). Intravenous Immunoglobulin Treatment for Repeated IVF/ICSI Failure and Unexplained Infertility: A Systematic Review and a Meta-Analysis. Am. J. Reprod. Immunol., 70(6), 434–447.

8- Singh, N., Davis, A. A., Kumar, S., & Kriplani, A. (2019). 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. Eur J Obstet Gynecol Reprod Biol., 240, 45–51.

9- Rinehart, J. (2007). Recurrent implantation failure: definition. J Assist Reprod Genet., 24(7), 284–287.

10- El-khayat, W., & El Sadek, M. (2015). Intralipid for repeated implantation failure (RIF): a randomized controlled trial. Fertil Steril., 104(3), e26.

11- Dakhly, D. M., Bayoumi, Y. A., Sharkawy, M., Gad Allah, S. H., Hassan, M. A., Gouda, H. M., Hashem, A. T., Hatem, D. L., Ahmed, M. F., & El-Khayat, W. (2016). Intralipid supplementation in women with recurrent spontaneous abortion and elevated levels of natural killer cells. Int J Gynaecol Obstet., 135(3), 324–327.

12- Akar, B., Doğan, O., Köle, E., & Çalışkan, E. (2022). Effect of Intravenous Lipid (SMOFLIPID®) Use along in Vitro Fertilization (IVF) Treatment in Women with Failed IVF Cycles Despite Good Quality Embryo Transfer. Acta Med Nicomedia, 5(3), 115-119.

13- Liu, S., Diao, L., Huang, C., Li, Y., Zeng, Y., & Kwak-Kim, J. Y. (2017). The role of decidual immune cells on human pregnancy. J Reprod Immunol., 124, 44–53.

14- Lédée, N., Vasseur, C., Petitbarat, M., Chevrier, L., Vezmar, K., Dray, G., Chenière, S., Lobersztajn, A., Vitoux, D., Cassuto, G. N., & Chaouat, G. (2018). Intralipid® may represent a new hope for patients with reproductive failures and simultaneously an over-immune endometrial activation. J Reprod Immunol., 130, 18–22.

15- Check, J., & Check, D. (2016). Intravenous intralipid therapy is not beneficial in having a live delivery in women aged 40-42 years with a previous history of miscarriage or failure to conceive despite embryo transfer undergoing in vitro fertilization-embryo transfer. Clin Exp Obstet Gynecol., 43(1), 14–15.

16- Rimmer, M. P., Black, N., Keay, S., Quenby, S., & Al Wattar, B. H. (2021). Intralipid infusion at time of embryo transfer in women with history of recurrent implantation failure: A systematic review and meta-analysis. J Obstet Gynaecol Res., 47(6), 2149–2156