International Journal of Gynecology and Obstetrics 135 (2016) 324–327

Contents lists available at ScienceDirect

International Journal of Gynecology and Obstetrics

journal homepage: www.elsevier.com/locate/ijgo

CLINICAL ARTICLE

Intralipid supplementation in women with recurrent spontaneous abortion and elevated levels of natural killer cells

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ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 13 June 2016
Accepted 21 August 2016

Keywords:
Intracytoplasmic sperm injection
Intralipid
In vitro fertilization
Natural killer cells
Recurrent spontaneous abortion

ABSTRACT

Objective: To investigate the efficacy of intralipid supplementation in women with recurrent spontaneous abortion (RSA) and elevated natural killer cell activity undergoing in vitro fertilization/intracytoplasmic sperm injection. *Methods:* Between February 10, 2013, and April 30, 2015, a double-blind randomized controlled study was conducted at a center in Egypt. Women with unexplained secondary infertility, RSA, and elevated levels of natural killer cells (>12%) were enrolled and randomly assigned to receive intralipid (2 mL diluted at 20% in 250 mL saline) or saline (250 mL) infusion on the day of oocyte retrieval using random numbers and sealed envelopes. Patients and attending physicians were masked to group assignment. The infusions were repeated within 1 week of a positive pregnancy test and then every 2 weeks until the end of the first trimester. The primary outcome was chemical pregnancy 14 days after embryo transfer. Analyses were by intention-to-treat. *Results:* Overall, 296 women were enrolled. Chemical pregnancy was recorded for 84 (58.3%) of 144 women in the intralipid group and 76 (50.0%) of 152 in the control group ($P = 0.129$). *Conclusion:* Intralipid supplementation did not increase frequency of chemical pregnancy. However, findings related to ongoing pregnancy and live birth should be investigated further.

ClinicalTrials.gov: NCT01788540

1. Introduction

The most common complication of pregnancy is spontaneous abortion: if preclinical pregnancy losses are included, approximately 50% of all human pregnancies have this outcome [1]. One-quarter to half of all couples experience at least one spontaneous abortion [1].

Recurrent spontaneous abortion (RSA) is known as three or more consecutive spontaneous abortions occurring before 20 weeks of pregnancy [2,3]. Primary RSA refers to three or more pregnancy losses before 22 weeks without a previous live birth, and secondary RSA refers to three or more pregnancy losses subsequent to a previous live birth or stillbirth [1]. RSA is mostly attributable to genetic abnormalities and autoimmunity [4]. Although some causes have been directly linked to parental chromosomal abnormalities, maternal thrombophilic disorders, structural uterine anomalies, or endocrine abnormalities, more than 50% of all occurrences of RSA are unexplained [5].

Adaptation of the maternal immune system—particularly the shift to type 2 T helper cells and the following decrease in function of type 1 T helper cells [6]—is critical for the maintenance of a successful pregnancy. Although many immunological disorders have been related to RSA, no definite immunological mechanism has been linked with RSA, with the exception of antiphospholipid syndrome. Consequently, many immunotherapies have been investigated with the aim of improving pregnancy outcomes.

One of the suggested therapies is intralipid, a fat emulsion containing soybean oil, glycerin, and egg phospholipids. Intralipid is used for parenteral nutrition in patients who cannot ingest food orally [7]. It is well known that parenteral fat emulsions accumulate in macrophages and cause impairment of their various functions [8]. Although the exact immune mechanism by which intralipid acts remains unknown, its active component, soybean oil, inhibits pro-inflammatory mediators, specifically type 1 T helper cells [9].

Natural killer (NK) cells are important constituents of innate immunity. They are found in both peripheral blood lymphocytes and the uterine mucosa. Approximately 50% of women with RSA have a moderate to marked increase in NK cell activity in their peripheral blood [10,11]. Various in vitro investigations have shown that intralipid has the ability to suppress NK cell activity [12]. Animal and human studies [13,14] indicate that intravenous intralipid could increase frequencies of ongoing pregnancy by improving implantation in patients with an elevated NK cell level. These findings indicate that intralipid could be a new effective therapeutic option for women with RSA and high levels of NK cells [15].

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http://dx.doi.org/10.1016/j.ijgo.2016.06.026
0020-7292/

The present study aimed to determine the efficacy of intralipid supplementation in women with RSA and elevated NK cell activity undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles.

2. Materials and methods

The present double-blind randomized controlled study was conducted at Kasr Al-Aini IVF Center, Cairo University, Egypt, from February 10, 2013, to April 30, 2015. Women with unexplained secondary infertility, three or more prior consecutive clinical spontaneous abortions after either a spontaneous pregnancy or IVF/ICSI, and an elevated level (>12%) of NK cells with CD16 expression or CD56 expression, or both, were enrolled. Women with RSA attributable to fetal chromosomal abnormalities (diagnosed by curettage and histopathological examination of the product of conception), antiphospholipid syndrome, or a positive thrombophilia test (antithrombin III or factor V Leiden deficiency, positive antinuclear antibodies or anti-double-stranded DNA antibodies, or positive antithyroid antibodies) were excluded from the study, as were those with uterine anatomical abnormalities (with the exception of an arcuate uterus), endocrine or medical disorders, parental chromosomal abnormalities, hyperlipidemia, or allergy to eggs or their products. Women older than 40 years were also excluded. The study was approved by the institutional review board before its start. All participating couples provided written informed consent.

Unexplained infertility was defined as normal ovarian reserve tests, normal semen analysis (according to the 2010 WHO criteria [16]), a normal uterine cavity, and patent fallopian tubes as detected by hysterosalpingography or laparoscopy before enrollment. The evaluation of NK cell function was performed by flow cytometry [17]. The cells were analyzed on an Elite XL flow cytometer (Beckman Coulter, Fullerton, CA, USA) collecting 10 000 events. As a control, unstained cells were applied first to exclude the effect of autofluorescence of the cells. The result was considered positive if the percentage of NK cells was higher than 12%.

After enrollment and during subsequent IVF treatment, participating women were randomly assigned to the intralipid group or the control group in a 1:1 ratio. Randomization was performed using computer-generated random number tables and sealed opaque envelopes. A nurse opened the envelopes just before oocyte retrieval, administered the assigned treatment, and concealed containers and intravenous lines so neither the patient nor the attending physician were aware of group assignment. Data analysts were also masked to group assignment.

Detailed history taking, a full systematic clinical examination with routine laboratory tests, and transvaginal sonography using a Voluson 730 Pro (GE, Fairfield, CT, USA) ultrasound machine were performed before the start of IVF treatment. The antral follicle count was measured and recorded on day 2–5 of the cycle. Preimplantation genetic screening was not performed for financial reasons.

A long agonist IVF protocol was used for all patients. Downregulation started on day 20 of the previous cycle using the gonadotropin-releasing hormone agonist triptorelin (0.1 mg Decapeptyl; Ferring Pharmaceuticals, Kiel, Germany). On the second day of menstruation, when downregulation was confirmed (as evidenced by endometrial thickness <5 mm and/or estradiol level <183.5 pmol/L), human menopausal gonadotropin (75 IU; Merional, IBSA, Lugano, Switzerland) was started at a daily dose of 225–300 IU intramuscularly, depending on the age and antral follicle count, while the triptorelin dose was reduced to 0.05 mg/day.

Follicular growth was monitored using serial ultrasonography examinations every other day starting from day 8 of human menopausal gonadotropin administration, and the dose was adjusted accordingly. When there were three or more leading follicles with a diameter of 18 mm or more, human chorionic gonadotropin (hCG; Choriomon, IBSA Institut Biochimique SA, Pambio-Noranco, Switzerland) was administered at a dose of 10 000 IU.

Ultrasonography-guided vaginal oocyte retrieval was performed 35 hours after hCG administration. Patients assigned to the intralipid group received a sealed intravenous infusion of 20% intralipid on the day of oocyte retrieval at a dose of 9 mg/mL of the total blood volume, corresponding to 2 mL intralipid (Frezenius, Clayton, NC, USA) diluted at 20% in 250 mL saline, over 30–60 minutes [15]. Patients assigned to the control group received a sealed intravenous infusion of 250 mL physiological saline solution.

A maximum of three embryos were transferred 3 days after oocyte retrieval. Any extra embryos were cryopreserved. Luteal phase support was given using progesterone vaginal suppositories (Cyclogest, Acatvis, Barnstaple, UK; 400 mg twice daily). The infusion (intralipid or saline) was repeated within 1 week of a positive pregnancy test and then every 2 weeks until the end of the first trimester.

The primary outcome of the study was chemical pregnancy, defined as a serum β-hCG concentration of more than 50 IU/L 14 days after embryo transfer. Secondary outcomes were clinical pregnancy, early spontaneous abortion before 12 weeks, and ongoing pregnancy at 12 weeks. Clinical pregnancy was defined as the presence of a positive heart beat in a healthy gestational sac 5 weeks after a positive β-hCG result. Post hoc outcomes assessed were live birth after 28 weeks and the implantation rate, which was calculated as the number of gestational sacs divided by the number of transferred embryos.

No sample size calculation was performed for the present study. Analyses were by intention-to-treat. The *t* test for independent samples was used to compare continuous variables. The χ^2 test was used to compare categorical data. $P < 0.05$ was considered statistically significant. All statistical calculations were performed using SPSS version 15 (SPSS Inc, Chicago, IL, USA).

3. Results

A total of 296 women were enrolled, underwent randomization, and received their assigned treatment (Fig. 1). There were no significant differences between the 144 women in the intralipid group and the 152 in the control group in terms of age, body mass index, number of previous spontaneous abortions, number of previous live births, number of previous failed cycles, number of oocytes collected, and number of transferred embryos (Table 1).

Chemical pregnancy (the primary outcome) was recorded in 84 (58.3%) women in the intralipid group and 76 (50.0%) in the control group ($P = 0.162$). The only significant differences in outcomes between the groups were the frequencies of ongoing pregnancy and live birth ($P = 0.005$ for both) (Table 2). A post hoc analysis showed that the present study had a power of 81.3% to detect differences between the two study groups in terms of the ongoing pregnancy rate and the live birth rate at an α level of 0.05.

No adverse effects of intralipid treatment were recorded.

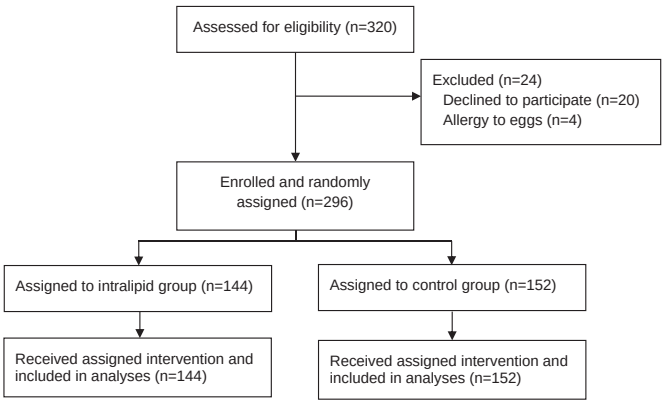

Fig. 1. Flow of participants through the study.

Table 1
Demographic and cycle characteristics.[a]

Characteristic	Intralipid group (n = 144)	Control group (n = 152)	*P* value
Age, y	35.5 ± 3.7	36 ± 3.7	0.390
Body mass index[b]	22.6 ± 2.6	22.9 ± 2.2	0.334
No. of previous spontaneous abortions	4.1 ± 0.9	4.1 ± 0.9	0.768
No. of previous live births	0.9 ± 0.7	0.9 ± 0.8	0.965
No. of previous failed cycles	2.1 ± 1.3	2.0 ± 0.9	0.436
No. of oocytes retrieved	8.5 ± 2.8	8.8 ± 2.4	0.481
No. of embryos transferred	2.5 ± 0.5	2.6 ± 0.5	0.196

[a] Values are given as mean ± SD unless indicated otherwise.
[b] Calculated as weight in kilograms divided by the square of height in meters.

4. Discussion

The present double-blind randomized controlled trial showed that intralipid administration to women from infertile couples with unexplained RSA and positive NK cell activity undergoing IVF/ICSI cycles increased both the ongoing pregnancy rate and the live birth rate. However, intralipid administration was associated with a nonsignificant increase in the chemical pregnancy rate and the clinical pregnancy rate, and a nonsignificant reduced in the spontaneous abortion rate.

The impact of intralipid on NK cell activation and cytotoxicity, and subsequent unexplained RSA has rarely been investigated. A review of the literature identified three previous studies in this area. The first study [12] demonstrated that intralipid, administered at a dose of 9–18 mg/mL, reduced NK cell activity against K562 target cells by 45.2% ± 8.3% in 275 women with RSA, measured using a flow cytometric method. The same group conducted a second study [15] showing that intralipid suppressed NK cell activity in vivo, and this suppression lasted for 6–9 weeks in 47 of 50 women with recurrent reproductive failure after IVF/ICSI cycles. However, the study did not include analysis of the effect on live birth rate. A more recent study [18] published in 2016 evaluated the efficacy of intralipid therapy among women aged 40–42 years with a previous history of spontaneous abortion or infertility undergoing ICSI. The study included a small number of patients (n = 4) with 10 matched cycles, and found that no clinical pregnancies were achieved in the intralipid group compared with a clinical pregnancy rate of 40% and a live birth rate of 30% in the control group. This previous study differed from the current investigation in that it was performed to detect the impact of intralipid on the live birth rate but it did not include or mention the number, percentage, or activity of NK cells, and it did not include women with unexplained RSA.

The association between NK cell activity and RSA has been investigated in a number of studies. A large systematic review published in 2011 [19] was conducted to detect the relation between NK cell activity and RSA. After collecting the information from 783 studies, only 12 were included. These studies were performed to detect whether an elevated percentage or activity of peripheral NK cells can predict future spontaneous abortion among patients with idiopathic RSA or implantation failure. The conclusion was that the prognostic value of the percentage or activity of peripheral NK cells is still uncertain, and larger studies were recommended. By contrast, a meta-analysis published in 2014 [20] outlined the relation of peripheral NK cells and RSA by pooling the data from four different studies, and found a significant difference in the activity of peripheral NK cells between women with RSA and those in the control group. However, pooled data from six studies showed no significant difference in the activity of uterine NK cells between the two groups [20].

Coulam et al. [21] showed that intralipid and intravenous immunoglobulins (IVIgs) are equally effective in reducing NK cell cytotoxicity and increasing the live birth rate among women experiencing RSA with elevated NK cell activity. However, there were no differences in the pregnancy and abortion rates between women treated with intralipid and age-matched women treated with IVIg. This also agrees with Nyborg et al. [22], who reported a clinical abortion rate of 9.6%, a live birth rate of 36.5%, and a cumulative live birth rate of 61.5% among patients with RSA undergoing IVF cycles and immunomodulation with IVIg and prednisone. Another meta-analysis [23] in women with secondary RSA showed a significant increase in the live birth rate after the use of IVIg.

The main limitation of the present study was the difficulty to find a reasonable number of women with unexplained RSA who were undergoing IVF/ICSI. To overcome this obstacle and recruit more women, the study duration was extended from 12 months to 26 months.

In conclusion, the present trial showed that intravenous infusion of intralipid is an effective means for increasing the ongoing pregnancy rate and the live birth rate in women with unexplained RSA undergoing IVF/ICSI cycles. However, it does not seem to increase the frequency of chemical pregnancy.

Table 2
Pregnancy outcomes.[a]

Outcome	Intralipid group (n = 144)	Control group (n = 152)	*P* value	Odds ratio (95% confidence interval)
Implantation	43 (29.9)	34 (22.4)	0.156	1.477 (0.876–2.491)
Chemical pregnancy	84 (58.3)	76 (50.0)	0.162	1.400 (0.884–2.215)
Clinical pregnancy	74 (51.4)	64 (42.1)	0.129	1.454 (0.918–2.299)
Spontaneous abortion	18 (12.5)	30 (19.7)	0.114	0.581 (0.307–1.096)
Ongoing pregnancy	54 (37.5)	34 (22.4)	0.005	2.082 (1.251–3.465)
Live birth	54 (37.5)	34 (22.4)	0.005	2.082 (1.251–3.465)

[a] Values are given as number (percentage) unless indicated otherwise.

Conflict of interest

The authors have no conflicts of interests.

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