ARTICLE

FERTILITY & REPRODUCTION

Intralipid Immunotherapy for Repeated IVF Failure

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ABSTRACT

The intravenous fat emulsion, intralipid, has been hypothesised to be an effective and safe treatment for repeated in vitro fertilisation (IVF), implantation failure and pregnancy loss. This exploratory, retrospective cohort study determined pregnancy outcomes and documented adverse events associated with intralipid use. Ninety-three women were identified as having received intralipid for a history of repeated unsuccessful IVF cycles and pre-viable pregnancy loss in two Australian IVF units that independently recruited between October 2014 and July 2016. Pregnancy outcomes and adverse events were recorded in fresh and frozen embryo transfer cycles in which the infusion was administered. The 93 women who received intralipid had a clinical pregnancy rate of 40.0%, compared with 35.0% in 651 age-matched controls undergoing IVF, which was not significantly different. The intralipid group had a livebirth rate of 35.7%. Apart from flushing, which was experienced by one individual, there were no adverse events associated with intralipid use. As a prelude to definitive evidence of benefit, we did not identify a safety concern or reduced pregnancy rates in intralipid users compared to controls. Indeed, these outcomes were better than expected in a poor prognosis group. This data supports an argument for large, randomised controlled trials to determine the benefit of intralipid in the treatment of recurrent implantation failure or miscarriage.

Keywords: Intralipid; IVF; Natural Killer Cells; Recurrent Implantation Failure; Immunotherapy; Miscarriage.

BACKGROUND

The success of in vitro fertilisation (IVF) is evidenced by the more than 6 million IVF babies born worldwide, its widespread availability and its applicability to almost any fertility problem. This conceals the reality that most IVF attempts do not result in pregnancy and success rates are less than 50% per cycle started. Aneuploidy likely accounts for the majority of unsuccessful IVF cycles (Donoso et al., 2007), however preimplantation genetic screening and transfer of only genetically normal embryos still only produces success rates of 60–70% (Lee et al., 2015). Given the significant physical, emotional and financial cost of each IVF cycle, therapies that improve implantation and pregnancy outcomes continue to be investigated. There is evidence that altered maternal immune function is a factor in recurrent implantation loss and miscarriage (Sacks, 2015a), although it is difficult to identify and define with current clinically available tests.

The clinical practice of reproductive immunology was dominated by Peter Medawar's seminal paper, describing the need for maternal immune suppression to accommodate an invading placenta (Sacks, 2015b; Medawar, 1953). However, it is now clear that some elements of the maternal immune system are activated and essential for successful implantation (Moffett and Colucci, 2014). Additionally, clinical trials demonstrate that immune suppressive therapy is not beneficial for most women undergoing IVF (Lee et al., 1994). There is ongoing debate that a subgroup of women with immune dysfunction may exist and benefit from immunotherapy. This group would be over-represented in the IVF population that experience recurrent implantation failure or recurrent miscarriage. More accurate immune testing is also proposed as a way of identifying this subgroup (Sacks, 2015a).

Immune therapies for disorders of placentation include lymphocyte immunotherapy (LIT), prednisolone, intravenous immunoglobulin (IVIG), and tumor necrosis factor-alpha antagonists (anti-TNFα) (Sacks, 2013). Results from published studies have not demonstrated a clear benefit from immunotherapy which could be attributed to poorly targeted patient groups (Clark, 2008) and/or poor study design. There is also concern that the expense and potential side effects of the therapies may outweigh the benefits (Robertson et al., 2016).

Historically, intralipid, a 20% fat emulsion comprised of soybean oil, egg phospholipids and glycerine, was initially used for parenteral nutrition, where its immunosuppressive effects were revealed by

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an elevated rate of bacteremia in neonates (Bansal et al., 2012). Intralipid was first suggested as a potential therapy for recurrent miscarriage in 1994 after it was used in the control arm of a double blinded randomised controlled trial in comparison to exposure to trophoblast membrane vesicles. Unexpectedly, a low miscarriage rate of 30% was reported in the 20 control patients (Clark, 1994; Johnson et al., 1991).

Intralipids ability to suppress blood natural kill cell cytotoxicity (Roussev et al., 2007; Roussev et al., 2008) was demonstrated in subsequent *in vitro* and *in vivo* studies in women. Ndukwe et al. (2011) reported a 50% livebirth rate with intralipid therapy in women with repeated IVF failure and immune dysfunction (Ndukwe, 2011).

The introduction of any new therapy requires caution, careful observation and transparent reporting of outcomes. At present intralipid must continue to be considered an empirical and experimental therapy. However, it is relatively inexpensive, is widely available, and poses no theoretical risk to a developing pregnancy. This retrospective observational study was designed to assess the impact of intralipid on pregnancy rates and determine the safety of intralipid therapy in women with repeated IVF failure or miscarriage as a preliminary step the exploration of its use for treating placentation disorders.

METHODS

Study design and inclusion criteria

Retrospective searching of clinical databases identified 93 women who received intralipid therapy at IVF Australia (IVFA) in Sydney, New South Wales and FertilitySA in Adelaide, South Australia. Sixty-eight women received intralipid at IVFA between October 2014 and May 2016, and 25 women received intralipid at FertilitySA between June 2011 and July 2016. The control group consisted of all age-matched patients at IVFA (n = 558) and FertilitySA (n = 93) who undertook IVF during the same time-period, identified on the clinical databases.

Livebirth was defined as the birth of a baby showing signs of life regardless of gestational age, and clinical pregnancy by fetal heart detection on ultrasound. These outcomes were recorded for all individuals. Adverse events related to intralipid therapy were identified from clinical notes. Other information obtained included demographic details, a fertility history, past medical history, and the type and results of testing undertaken for implantation disorders. The pregnancy outcomes of individuals receiving intralipid were correlated to demographic indices and tests for implantation failure and miscarriage to attempt to identify a subgroup of women most likely to benefit from intralipid therapy.

The analysis of pregnancy outcome data was confined to women who had an embryo transfer in the context of an IVF cycle. Women who conceived naturally were excluded from analysis of pregnancy outcomes as most received intralipid after conception. We were not able to obtain the livebirth outcome for 1 woman who received intralipid but relocated overseas during her pregnancy.

Immune testing

Assays for anti-phospholipid antibodies, anti-nuclear antibodies and anti-thyroid antibodies were performed by commercially available automated laboratory kits (Douglass Hanly Moir, Sydney; SA pathology and Clinpath, Adelaide). Lupus anticoagulant, Kaolin Clotting time and thrombophilias were also tested using commercial assays (Douglass Hanly Moir, Sydney; SA pathology and Clinpath, Adelaide). High levels of peripheral blood natural killer (pbNK) cells were defined as pbNK cells comprising >18% of total lymphocytes with activated cells ($CD56^{dim}CD69^{+}$) at a concentration $>12 \times 10^6$/mL (King et al., 2010; Sacks et al., 2012). pbNK cell levels were defined as borderline when only one of the above criteria was met, or if the percentage of pbNK cells was 12–18%. Normal levels were <12% of total lymphocytes and a concentration of $CD56^{dim}$ $CD69^{+}$ cells $<12 \times 10^6$/mL. Elevated levels of uterine natural killer (uNK) cells were defined as the percentage of CD56 immuno-positive staining being above the >75th percentile of normalised population results on a given day of the menstrual cycle (Russell et al., 2011). This was determined by immunohistochemical staining for CD56 and morphometric analysis of luteal phase endometrial tissue sections.

Intralipid protocol

Intralipid 20% (Intralipid®, Fresenius Kabi, Hamburg, Germany) was given as an intravenous infusion made up from 100 mL of intralipid diluted in 500 mL of normal saline over 3 to 4 hours in a clean, sterile inpatient hospital environment. Women at IVFA received an intralipid infusion on day 5–9 of their cycle, and again following detection of a positive ß-HCG. FertilitySA women received intralipid infusion at oocyte retrieval in a fresh embryo transfer cycle or at embryo transfer when in a frozen embryo transfer cycle, and then again at detection of a positive ß-HCG. A positive ß-HCG was defined as a serum estimate >25IU.

Statistical analysis

Descriptive statistics are presented as median and range. An unpaired *t*-test was used to compare the mean age of women who fell pregnant with those who did not. Pearson's chi-squared test was used to measure the correlation between pregnancy rate with prenatal genetic testing for aneuploidy (PGT-A), history of recurrent miscarriage and participation in the donor egg program. Goodman and Kruskal's gamma test was used to measure the correlation between NK cell levels (both pbNK and uNK) and pregnancy rate, as well live birth rate. Statistical significance was considered to be reached at a p value of 0.05. The statistical analysis was performed using SPSS 23.0.

RESULTS

Patient characteristics

A total of 93 women received intralipid therapy. Patients receiving intralipid had an average of 4.7 years of infertility, 2 previous miscarriages, 5 previous oocyte retrievals and 7 previous embryo transfers characteristic of a poor prognosis group of patients. Endometriosis (62%) was the most common factor that impacted fertility, with a low ovarian reserve (42%) the next most frequent. The demographic and fertility characteristics of women receiving intralipid are presented in Table 1.

High levels of pbNK (77%) or uNK cells (44.6%) were the most common immune alteration seen in those tested. Elevated anti-nuclear and anti-phospholipid antibodies were identified in 38% and 24.7% respectively of those tested. The results of immune and coagulation testing are presented in Table 2.

Intralipid cycles

Of 93 women, 85 underwent an embryo transfer (ET) during their cycle with intralipid infusion. Four women were given intralipid before oocyte retrieval in a cycle that was cancelled and 4 women with a history of recurrent miscarriage used intralipid with a positive ß-HCG in a spontaneous conception. The most common cycle type was a frozen embryo transfer (n = 51), followed by ICSI (n = 23) and IVF (n = 10). In 30 of these cycles, PGT-A was used to ensure a chromosomally normal embryo was transferred. Other immune modulating agents including prednisolone, clexane, heparin, aspirin and the endometrial scratch were used in 58 women. Most women used their own oocytes (n = 73), while 12 used donor eggs. Sixty-seven

Table 1. Demographic and fertility characteristics.

Female infertility history	**Median (range)**
Age at time of intralipid infusion (years)	39 (24–51)
Duration of infertility (years)	4.7 (1.1–13.7)
Previous miscarriages	2 (0–8)
Previous prior egg collections	5 (0–15)
Previous number of embryos transferred	7 (0–22)
Number with previous livebirth (%)	17 (18.3%)
Factors that impact fertility	**No. positive/Total tested (%)**
Abnormal karyotype	3/84 (3.6)
BMI >25	16/54 (29.6)
Endometriosis[a]	31/50 (62.0)
Tubal obstruction or salpingectomy	17/58 (29.3)
Uterine fibroids	22/87 (25.3)
Past history of uterine polyps	14/90 (15.6)
Past history of uterine septum	8/87 (9.2)
Hypothalamus-pituitary dysfunction	5/57 (8.8)
Polycystic ovarian syndrome[b]	14/87 (16.1)
Low ovarian reserve[c]	36/84 (42.9)
Past history of abnormal thyroid function tests[d]	16/89 (18.0)
Male partner sperm quality	**Abnormal/Total tested (%)**
Semen analysis[e]	35/55 (63.6)
Sperm Chromatin Structure Assay[f]	7/33 (21.2)

The percentage positive is the number with a positive test/ the total number of patients who had the test performed.
a. Endometriosis visually identified at laparoscopy
b. PCOS defined by Rotterdam criteria
c. Anti-Mullerian hormone <10
d. 8/16 were euthyroid on thyroxine, 4/16 had a history of subclinical hypothyroidism, 4/16 were euthyroid with antibodies
e. WHO criteria — any abnormality in concentration, motility and/or morphology
f. DNA damage >15%

Table 2. Immunological and thrombophilia characteristics.

NK cell assays	**No. positive/Total tested (%)**
Elevated pbNK cells	47/61 (77.0)
High levels	12/61 (19.7)
Borderline levels	35/61 (57.4)
Elevated uNK cells	29/65 (44.6)
Other immunological assays	**No. positive/Total tested (%)**
Anti-phospholipid antibodies	19/77 (24.7)
Positive Kaolin clotting time	6/25 (7.5)
Anti-nuclear antibodies	19/50 (38.0)
Anti-thyroid antibodies	9/61 (14.8)
Thrombophilia Screening	**No. Positive/Total tested (%)**
Homozygous or compound heterozygous MTHFR	15/80 (18.8)
Elevated homocysteine	9/80 (11.3)
Factor V Leiden	4/80 (5.0)
Other*	4/80 (5.0)

The percentage positive is the number with a positive test/ the total number of patients who had the test performed.
* Elevated protein S, low anti-thrombin 3, activated protein C resistance, heterozygous prothrombin.

women underwent only one cycle with intralipid, 13 undertook a second cycle, and a further 5 women had 3 or more cycles.

Pregnancy outcomes

There were 44 pregnancies in 85 women who had an intralipid infusion during an embryo transfer cycle. A fetal heart was detected on ultrasound in 34 of these pregnancies, while 10 had an early miscarriage. This translates to a clinical pregnancy rate of 40.0%, compared to 35.0% in the control group, which was not significantly different. Of the 34 clinical pregnancies, 3 miscarried after fetal heart was detected, 30 resulted in livebirth, while 1 was lost to follow-up. This translates to a livebirth rate of 35.7% (Fig. 1). Although a higher percentage of clinical pregnancies were seen in subsequent intralipid cycles the numbers of women in the groups are low and more data is needed to remove uncertainty from this finding. Forty-nine per cent of those who underwent only 1 cycle had a clinical pregnancy, 52% of those who underwent 2 cycles and 80% of those who underwent 3 or more cycles became pregnant. A further 7 women achieved a clinical pregnancy following their intralipid therapy, either naturally or during a subsequent IVF cycle.

Correlations with positive pregnancy

Sub-group analysis revealed a statistically significant ($p = 0.03$) relationship between positive pregnancy test after intralipid and pbNK cells, with a greater proportion of women with borderline and high levels of pbNK cells having a positive pregnancy test after intralipid than those with normal pbNK cell levels (Fig. 2). There was a similar but non-significant ($p = 0.095$) trend to higher livebirth in intralipid treated women with elevated levels of pbNK cells, compared to those with borderline or normal pbNK cells (Fig. 3). No correlation was found between pregnancy rate and uNK cell levels, PGT-A, the number of previous miscarriages or when donor eggs were used (Table 3).

In the IVFA group, no difference ($p = 0.806$) was found in age between the women who fell pregnant (mean of 38.5 years ± 4.7) and those who did not (38.8 years ± 4.6). In the FertilitySA group, those who fell pregnant were younger (mean age 36.2 ± 4.2) compared with those who did not (mean age 40.9 ± 4.4), at a statistically significant level ($p = 0.024$). When combined, women who fell pregnant were younger (mean 37.8 years ± 4.8) compared with those who did not (mean 39.24 years ± 4.7), but this was not statistically significant ($p = 0.164$). Overall, 38.6% of the women who fell pregnant were aged over 40 years ($n = 17$) and only four of these women used donor eggs.

Safety outcomes

One patient with a history of seizures suffered a "pre-seizure, flushing" sensation. One gestation was complicated by asymmetrical intrauterine growth restriction. Other adverse pregnancy outcomes were an adherent placenta in 1 patient, as well as 3 cases of prematurely delivered dichorionic diamniotic twins, all of whom were conceived in cycles where two embryos were transferred.

DISCUSSION

This study is the first analysis of pregnancy outcomes and safety after intralipid therapy in Australia. In a group of women with a history of extremely poor fertility outcomes, the livebirth rate of 35.7% and clinical pregnancy rate of 40.0% after intralipid infusion was comparable to the control clinical pregnancy rate of 35.0% and did not indicate a reduction in pregnancy rates with intralipid use. We demonstrated that intralipid administration was associated with few adverse events. Our data indicates that intralipid is safe and does not hinder conception, in women with repeated IVF failure and recurrent miscarriage. This study was not designed to prove benefit of intralipid treatment.

Research into the use of intralipid in reproductive disorders has been limited. A proof-of-concept study revealed significantly lower abortion rates in mice treated with intralipid (Clark, 1994). Published only in abstract form, a randomised control trial for

Fig. 1. Flow diagram of the pregnancy outcomes of women who received and intralipid infusion and had an embryo transfer. Fifty-one per cent had a positive pregnancy test, 11% had a biochemical pregnancy and 40% had a clinical pregnancy. Thirty livebirths were recorded with 3 pregnancies resulting in miscarriage. One patient relocated overseas and was lost to follow-up.

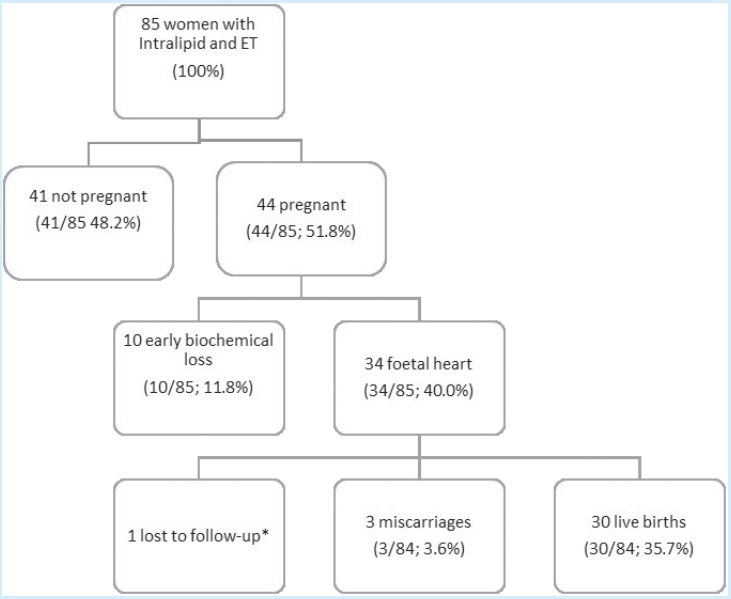
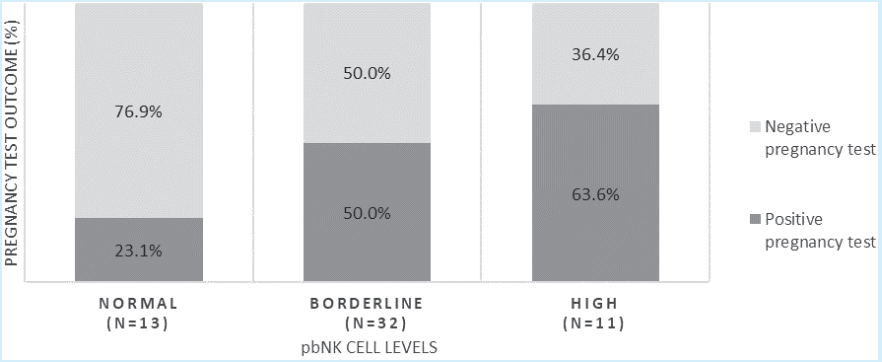


Fig. 2. Pregnancy rate stratified by pbNK cell levels. Rank correlation between levels of pbNK cells and pregnancy test reached statistical significance (p = 0.03). A gradient of clinical benefit based on pbNK cell levels was seen (those with the highest pbNK levels demonstrated the most benefit from intralipid infusion).



women with recurrent miscarriage demonstrated a significantly higher livebirth rate (33%) in women treated with intralipid therapy (n = 101) compared with a 13% livebirth rate in the control group (n = 102) (El-khayat and El Sadek, 2015). Similarly, in 296 women with recurrent implantation failure and abnormal pbNK cell levels, those randomised to intralipid had a significantly higher livebirth rate of 37.5% compared to 22.4% in the control group that received normal saline (Dakhly et al., 2016). Our livebirth rate of 35.7% in the intralipid group is consistent with the findings from these studies.

Two studies reported no pregnancies in women aged over 40 years treated with intralipid therapy, and suggested there may be no treatment benefit in this group (Acacio et al., 2008; Check and Check, 2016). These studies were underpowered to detect a true effect as few women of advanced maternal age were treated (n = 4 and n = 10, respectively). In our study, 38% of the women (n = 17) who had a clinical pregnancy were aged over 40 at the time of intralipid infusion and none suffered an adverse event. Our data provides evidence that intralipid did not impact negatively on pregnancy outcomes or safety in women of advanced maternal age.

Fig. 3. Livebirth rate stratified by pbNK cell levels in women who became pregnant and underwent pbNK cell testing. Rank correlation reached p = 0.095.

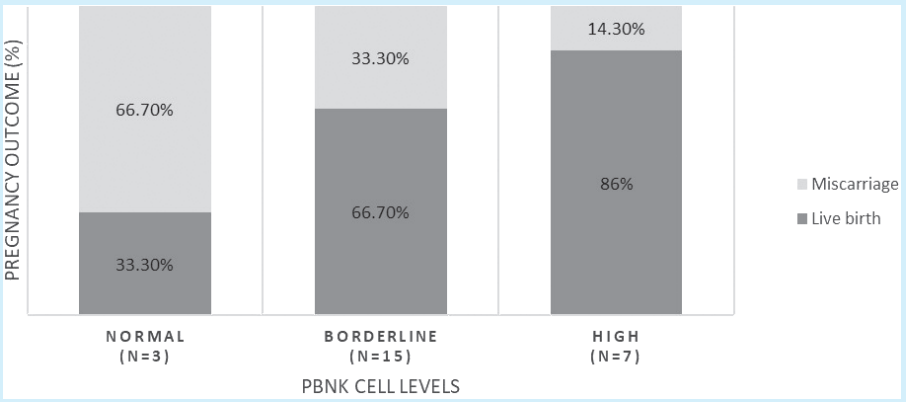


Table 3. Pregnancy rate based on uNK cell levels, PGD testing and number of miscarriages.

Parameter	Factor	No. pregnant/Total with factor (%)	P value
uNK cell levels	Normal	16/34 (47.1)	0.519
	Elevated	16/29 (55.2)	
PGD testing	PGD tested	16/30 (53.3)	0.846
	Not PGD tested	24/47 (51.1)	
Number of previous miscarriages	< 3 miscarriages	25/48 (53.1)	0.654
	≥3 miscarriages	16/34 (47.1)	
Egg type	Own	38/73 (52.1)	0.895
	Donor	6/12 (50.0)	

The low rate of adverse events seen in our study suggests that intralipid is a safe immunomodulatory agent and is consistent with other recent trials that reported no adverse effects (Meng et al., 2016; Dakhly et al., 2016). This is reassuring given a recent report of three cases of sepsis following intralipid infusions in the UK (RCOG letter from president, 10/4/15). Intralipid is a nutritious and appealing substance for pathogens, and its administration in a sterile, clinical environment is critical to prevent infection. Intralipid also has the potential to cause minor adverse effects including headache, dizziness, flushing, drowsiness, nausea, vomiting and sweating. In our study, only one woman with a history of seizures complained of a flushing, pre-seizure sensation during infusion, suggesting that these complications are rare.

Intralipid has low teratogenic potential and no congenital abnormalities were identified in women who had received intralipid in our study. Of the 30 livebirths to babies of mothers who had received intralipid, there was only 1 case of asymmetrical intrauterine growth restriction. It is unlikely that the reported adverse pregnancy outcome was related to intralipid infusion. Reassuringly, intralipid seemed tolerable and acceptable to women with recurrent miscarriage and recurrent implantation failure, given that 19 women willingly underwent repeated cycles with intralipid and 44 women who fell pregnant tolerated a second infusion.

Due to a variety of factors related to their IVF cycles, 4 women underwent intralipid infusions in the follicular phase without subsequent embryo transfer. These women were exposed to the risks and costs of intralipid infusion without a potential benefit, which is less than ideal. As intralipid was administered in either the follicular phase or at the time of oocyte retrieval with no demonstrable difference in pregnancy rates, it would be reasonable to administer the infusion at time of oocyte retrieval to avoid its use in cancelled cycles.

Clark argues that the efficacy of a treatment can only be truly assessed in patients identified to have a specific condition that the therapy is intended to target (Clark, 2008). Ideally, selected women at risk would be identified from a clinical history and given well-defined tests for placental immune dysfunction. Those with a proven immune aberration would then be offered selective treatment with intralipid, after it has been shown to have significant benefit in a series of randomised control trials.

Unfortunately, a clinical history or recurrent implantation failure and miscarriage identifies a very heterogeneous group of women, and immune disorders are only one of many genetic, metabolic and medical factors associated with these conditions (Franasiak and Scott, 2017). In our study, several women had known factors that impact on implantation, including endometriosis, fibroids and intrauterine pathologies which could have confounded the findings.

Some tests, such as preimplantation genetic screening or karyotyping of products of conception may be accurate in diagnosing the cause of implantation or pregnancy failure. However, other tests for placental immune dysfunction have much lower sensitivities and specificities (Hviid and Macklon, 2017), leading to diagnoses such as seronegative antiphospholipid syndrome (Nayfe et al., 2013) and the rise of empirically based treatments (Sacks, 2015a). Another fallout of poor testing is the inability to select patients for randomised controlled trials in a way that accurately tests immunomodulatory therapies without confounding effects from genetic and non-immune related causes of implantation failure and pregnancy loss (Clark, 2008). Sub-categorisation with better immune testing and more targeted treatments for specific groups of patients would significantly advance developments in the field.

In our study, a significantly greater proportion of women became pregnant after intralipid therapy when elevated pbNK cells were present, than when women with normal levels received an intralipid infusion. This correlation was not seen when uNK cells or other markers of immune dysfunction were measured. Several studies have demonstrated an association between elevated numbers or activity of pbNK cells and pregnancy loss (Beer et al., 1996; Coulam and Roussev, 2003; Matsubayashi et al., 2005; Sacks et al., 2012), although others suggest that elevated pbNK cells have no predictive

value in pregnancy outcomes (Moffett et al., 2004; Thum et al., 2005). Intralipid infusions have been demonstrated to reduce peripheral blood natural kill (pbNK) cytotoxicity in vitro (Roussev et al., 2007) and in vivo (Roussev et al., 2008). In 68 women with elevated pbNK activity and a history of recurrent implantation failure treated with intralipid, a 40% pregnancy rate was recorded (Acacio et al., 2008). Our paper neither correlated a reduction in pbNK cytotoxicity with improved pregnancy outcomes, nor measure pbNK cell levels after intralipid therapy.

Roussev et al.'s found that some women required 2 or 3 intralipid infusions to return elevated pbNK cell levels to normal values (Roussev et al., 2008). This may have some weak support from the suggestion that women in our study were more likely to fall pregnant in their second or third intralipid cycles than in their first, although the numbers in these subgroups are too low to provide certainty. It is possible that intralipid-mediated alterations in pbNK cell activity is a potential mechanism of action. Future studies correlating changes in pre- and post-infusion pbNK levels with pregnancy outcomes would improve our understanding of the biological basis of intralipid's effect and may help identify a way of selecting a group of patients who would benefit from intralipid treatment.

In a prospective randomised study, equivalently high pregnancy rates were reported when IVIG and intralipid treatments were compared in 154 women with recurrent miscarriage and elevated pbNK cells (Meng et al., 2016). IVIG costs 5 times more than intralipid, and has known side effects including headaches, skin rashes and nausea, with more severe adverse events like anaphylaxis, renal failure and thromboembolism occasionally reported (Moffett and Shreeve, 2015). Thus, intralipid appears to be a safe, cost-effective alternative to IVIG use for women with recurrent miscarriage.

The most effective protocol for intralipid has not been established and we have yet to determine the optimum number of intralipid infusions, the ideal timing of these infusions, the benefits or harms of using intralipid in isolation or combination, and whether intralipid should be given as a pregnancy support post-conception. Our study sheds some light on these issues, albeit our findings are limited by small numbers. At both sites, an infusion was given during an ART cycle and at the time of a positive pregnancy test, and the slightly different timing of infusions did not appear to impact on pregnancy outcomes.

In our study, 58 women used intralipid with another therapy, revealing clinical decisions to use intralipid in combination. This study is not able to separate the impact of individual therapies used concurrently with intralipid as small subgroup numbers prohibited further analysis and a future risk-benefit analysis of combined use is critical. Although some benefit from combinations of IVIG with anti-TNFα (Winger and Reed, 2008; Winger et al., 2009) and IVIG with prednisolone (Nyborg et al., 2014) have been proposed, the potential consequences of excessive immunosuppression may impact negatively on conception and placentation (Robertson et al., 2016) and can be harmful, as demonstrated by a case report detailing a fetal loss at 18 weeks from systemic candidiasis following administration of a combined protocol of intralipid with prednisolone and anti-TNFα (Akhanoba et al., 2014).

Three women received intralipid after conceiving naturally and went on to deliver a live born child, raising the possibility of an alternative use for intralipid in preventing miscarriage. If intralipid proves effective in this scenario, some couples may be able to avoid IVF and its associated burdens as a treatment for recurrent miscarriage.

The major limitations of the present study are its observational, retrospective design and small size. However, observational studies play a critical role in the progress of research, especially when so little is known, as is the case with intralipid. At the very least, this study demonstrates a need for future research into intralipid, and provides direction for the design and allocation of resources (Clark, 2009). At present, it appears that intralipid is safe and not associated with poorer pregnancy outcomes in women. Our findings support the possibility that intralipid could enhance pregnancy outcomes, particularly in women with markers of immune dysfunction. Future large, prospective studies with a well-matched control group are needed to further elucidate the utility of intralipid's use in reproductive medicine and to direct future clinical practice.

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CONFLICTS OF INTEREST

MLH has a private fertility practice (Embrace Fertility) in Adelaide, Australia. GS is a shareholder in IVFAustralia and has a private fertility practice in Sydney, Australia.

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